



المؤتمر الوطني العاشر للبيئة والموارد الطبيعية

29-30 تشرين الأول 2025

**The Tenth National Conference for the
Environment and Natural Resources**

Conference

29-30 October 2025, Basrah, Iraq.

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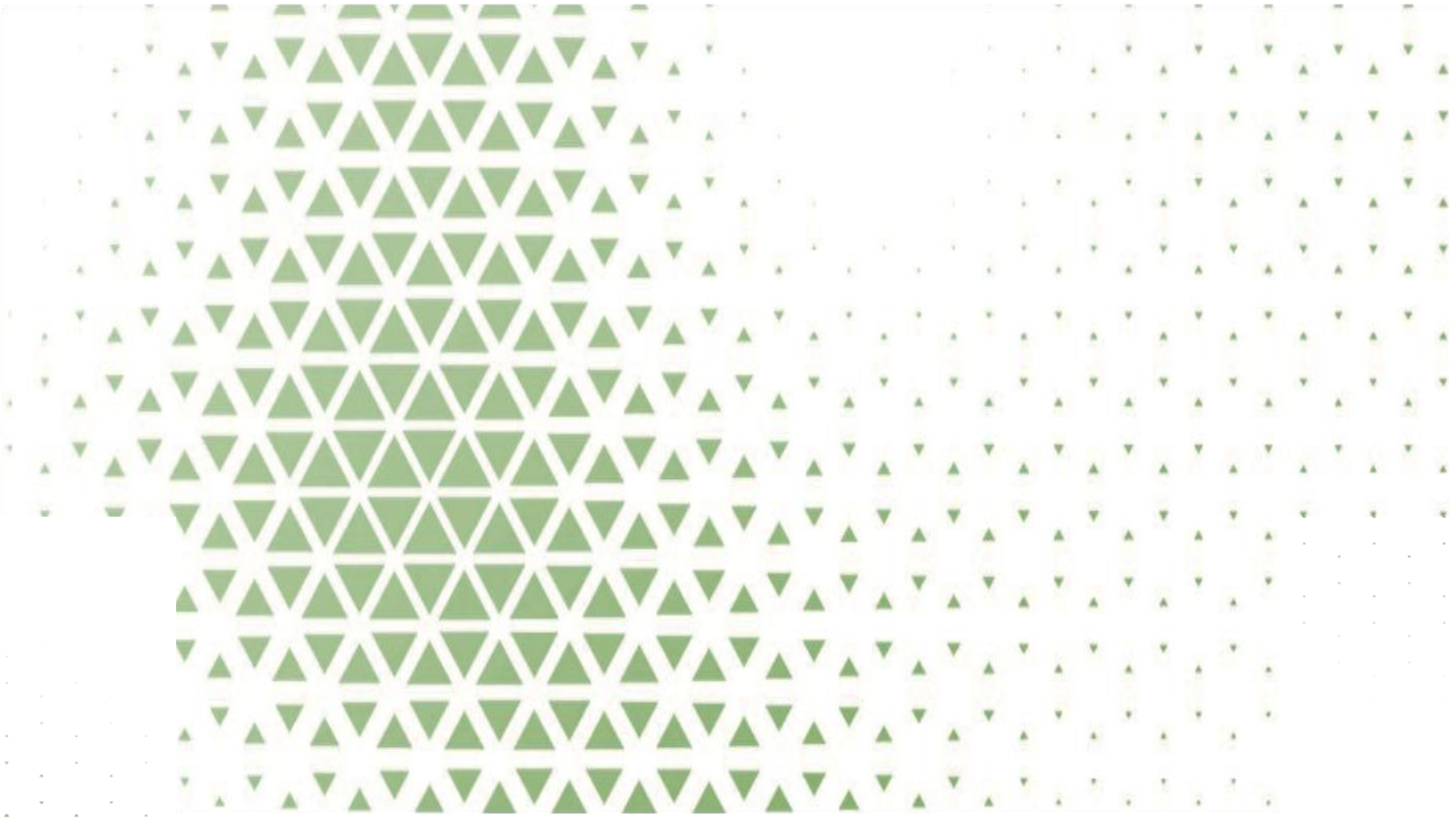
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Seasonal Variation of Residual Trace Metal Distribution and Sources in Oil Fields in Basrah, Southern of Iraq

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Abstract. Soil samples were collected seasonally (summer, autumn, winter, and spring) from 11 oil fields: Majnoon, Ratawi, Al Zubair, West Qurna 1, West Qurna 2, South Rumaila, North Rumaila, Shuaaba, Safwan, Seba, and Bergezia. At each site, soil samples were analyzed for Residual heavy metals (Zn, Cd, Mn, Pb, Fe, Cu and Cr) using Inductively Coupled Plasma (ICP) analysis. The study used indicators such as the Contamination Factor (CF), Enrichment Factor (EF), Geo-Accumulation Index (Igeo), and Pollution Load Index (PLI) to assess the contamination levels. The concentrations of trace metals: Zinc (Zn) had highest value (83.217 µg/g dry weight in the Al-Zubair oil field in autumn) and lowest value: (0.009 µg/g dry weight in the Bergezia oil field in spring). Cadmium (Cd) had highest value (1.995 µg/g dry weight in the Seba oil field in winter) and lowest value (0.001 µg/g dry weight in Al-Zubair and Bergezia oil fields in Spring). Manganese (Mn) highest value (526.347 µg/g dry weight in West Qurna 2 oil field – Autumn) and lowest value (0.008 µg/g dry weight in Bergezia oil field in Spring). Lead (Pb) highest value (39.288 µg/g dry weight in West Qurna 2 oil field in Summer) and lowest value (0.022 µg/g dry weight in Bergezia oil field in Spring). Iron (Fe) highest value (9676.116 µg/g dry weight in Seba oil field in Spring) and lowest value (1.263 µg/g dry weight in Bergezia oil field in Spring). Copper (Cu) highest value (304.929 µg/g dry weight in West Qurna 1 oil field in Summer) and lowest value (0.001 µg/g dry weight in Bergezia oil field in Spring). Chromium (Cr) highest value (89.351 µg/g dry weight in Ratawi oil field in Winter) and lowest value (0.101 µg/g dry weight in Bergezia oil field in Spring).

Keywords. Basra oil fields, Residual, Trace metals , Soil, Seasonal.

1. Introduction

Natural oil systems are globally prevalent. In Iraq, the southern city of Basrah has been a focal point for substantial oil exploration, extraction, and exportation activities carried out by the national oil industry for many years [1]. Basrah is located at the junction of the Shatt Al-Arab River, which supplies fresh water and is anticipated to receive drainage water, as well as the Arabian Gulf [2], where return flows from oil refineries and contaminated wastewater are expected to be directed [3]. The climate in Basrah is notable for its extreme heat and humidity during the summer months, whereas winters are relatively mild. Approximately half of Basrah's area is recognized as one of the world's key oil-producing regions [4]. Trace metals occur naturally in the environment and are integral components of biological tissues. Nonetheless, certain trace metals can become toxic at high concentrations [5]. These metals enter the environment via natural terrestrial sources and anthropogenic activities. Human-related sources of trace metal contamination include mining and ore extraction, smelting processes, activities associated with fossil fuel industries, waste disposal operations, agricultural practices, and waste incineration [6]. Contaminated solid particles, such as dust and soil accumulate trace metals, which can subsequently alter the chemistry of both freshwater and marine ecosystems [7]. Additionally, contaminated soils can lead to bioaccumulation of trace metals in crops, posing risks to human health through the consumption of affected crops[8]. Consequently, concerns regarding contaminated soils are significant from both environmental and public health perspectives. These issues underline the importance of studying solid matrices including oils, soils, sediments, and suspensions [9]. The Aims of the Study determine the concentration, sources, and origins of residual trace metals in the soil of 11 oil fields in Basrah, southern Iraq, and determine the regional and seasonal variation of these metals in the soils of oil fields.

2. Materials and Methods

2.1. Study Area

Soil samples were collected from 11 locations within the oil fields of Basrah City (Majnoon, Ratawi, Al Zubair, West Qurna 1, West Qurna 2, South Rumaila, North Rumaila, Shuaaba, Safwan, Seba, and Bergezia) as shown in Figure(1). Sampling was conducted at a depth of 20cm, between August 2024 and April 2025.

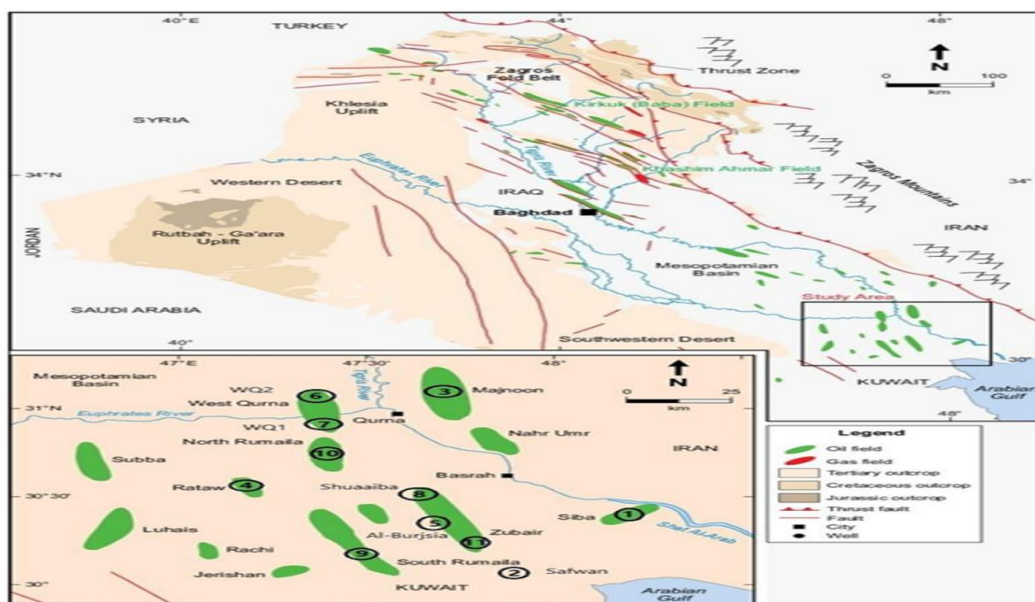


Figure 1. Samples locations of The Study Area.

2.2. Heavy Metals Analysis

2.2.1. The Residual

The residual heavy metals were extracted using [10] concentrated HCl and HNO₃(1:1) was added to each sample and evaporated to near dryness on a hotplate at 80°C, then a mixture of concentrated HClO₄ and HF (1:1) was added. After heating to near dryness, 20 ml of 0.5N HCl were added and cooled for 10 min. The extraction was decanted into a 25 ml plastic volumetric flask. This step was repeated twice and all supernatant were combined. Samples were stored in tight stopper polyethylene vials for analysis using inductively Coupled Plasma (ICP).

2.2.2. Determination of Contamination Factor (CF)

The contamination Factor was used to determine the contamination status of the soil in this study. The CF was calculated using the following equation:

$$CF = M_c / B_c$$

Where...

M_c: The measured concentration of the metal.

B_c: The background concentration of the same metal.

Table 1. The classification of contamination factor [11].

CF	Indicate of Contamination Factor
CF < 1	low contamination
1 ≤ CF ≤ 3	moderate contamination
3 ≤ CF < 6	considerable contamination
CF > 6	very high contamination

2.2.3. Determination of Enrichment Factor (EF)

To evaluate the source material found in Earth's crust [12], EF was calculated using the equation following:

$$EF = (CM / CFe)_{\text{sample}} / (CM / CFe)_{\text{Earth's crust}}$$

Where,

(CM/CFe): sample ratio of trace metal concentration.

(CM/CFe) Earth's crust: is the same reference ratio in the Earth's crust; the reference value of Fe is 5.2% and was selected as the reference element, because of its crustal dominance and high immobility.

Table 2. The classification of enrichment factor(EF) [12].

EF	Indicates of Enrichment Factor
EF <1	no enrichment
EF = 1-3	minor enrichment
EF = 3-5	moderate enrichment
EF = 5-10	moderate to severe enrichment
EF = 10-25	severe enrichment
EF 25-50	very severe enrichment
EF >50	extremely severe enrichment

2.2.4. Determination of Geo Accumulation Index (Igeo)

Geo accumulation index (Igeo) values were calculated for different metals, (Igeo) using the equation introduced by [13] as follows:

$$I\text{-geo} = \log_2 (C_n / 1.5 B_n)$$

Where,

Cn: The measured concentration of element n in the soil.

Bn: The geo accumulation background for the element n.

Table 3. The classification of (Igeo) [13].

Igeo	Soil Pollution Case
<1	practically unpolluted- Background sample
1-2	unpolluted to moderately polluted
2-3	moderately polluted to polluted
3-4	strongly polluted
4-5	strongly to extremely polluted
>5	extremely polluted

2.2.5. Pollution Load Index (PLI)

Soil pollution load index was calculated using the equation:

$$PLI = \sqrt[n]{(CF1 \times CF2 \times CF3 \times \dots \times CFn)}$$

Table 4. The classification of (PLI) [14].

PLI	The Indicates
value > 1	Pollution
value < 1	no pollution

3. Results and Discussion

The seasonal variation in the concentration of residual heavy metals revealed notable differences across the four seasons, reflecting the dynamic nature of environmental and anthropogenic influences, As shown in Tables(5,9,13,17) and Figure(2,3,4,5). Overall, the highest concentrations of most metals were observed during the autumn and winter seasons, whereas spring generally recorded the lowest values for most elements. This pattern may be attributed to factors such as seasonal industrial activities, atmospheric deposition rates, and variations in precipitation and temperature, all of which affect metal mobility and accumulation in soils [15,16].

Zinc (Zn) concentrations were highest in autumn [17], with values reaching up to 83.217 $\mu\text{g/g}$ in Al Zubair, while the lowest values were observed in spring, dropping to 0.009 $\mu\text{g/g}$ in Bergezia. This seasonal discrepancy may be linked atmospheric deposition during the post-summer period [15]. A similar pattern was observed for Cadmium (Cd), where autumn exhibited a peak value of 1.815 $\mu\text{g/g}$ and spring reported extremely low levels, suggesting that temperature and moisture conditions may play a role in Cd retention and migration [18].

Manganese (Mn), a naturally abundant element, was elevated in autumn and summer, with maximum concentrations reaching 526.347 $\mu\text{g/g}$ in West Qurna2 in autumn, likely due to enhanced soil oxidation processes. In contrast, spring showed significantly reduced levels, indicating to limited microbial activity and lower mineralization rates at cooler temperatures [19].

For Lead (Pb), the seasonal trend showed the highest concentrations during winter, especially at Seba (17.852 $\mu\text{g/g}$), possibly because of increased fuel combustion and vehicular emissions during colder months. In spring, the values dropped significantly, reinforcing the link between atmospheric input and seasonal usage patterns of fossil fuels [20,21].

Iron (Fe) followed a similar seasonal distribution, with the maximum level observed in winter (9279.445 $\mu\text{g/g}$ at West Qurna1) and the minimum in spring (1.263 $\mu\text{g/g}$ at Bergezia). This could reflect industrial activity, while limited mobility during spring may restrict Fe distribution [22].

Copper (Cu) displayed a highly variable pattern, peaking at 304.92 $\mu\text{g/g}$ in West Qurna1 during summer, likely due to agricultural applications or industrial inputs. The lowest levels were recorded during spring, coinciding with low biological activity and possibly enhanced leaching [17].

Finally, Chromium (Cr) concentrations peaked in spring at 83.490 $\mu\text{g/g}$ in Seba, in contrast to its low point during summer at Al Zubair (0.008 $\mu\text{g/g}$). The elevated spring levels may stem from historical deposition or geogenic sources mobilized during the seasonal transition phases [23].

Collectively, these findings indicate that the residual heavy metal distribution in soil is strongly influenced by seasonal variations, with autumn and winter posing higher contamination risks. These seasonal dynamics should be factored into environmental monitoring programs and risk assessment frameworks to better manage the ecological impacts of heavy metal accumulation. The analysis of Trace Element concentrations based on CF, EF, Igeo, and PLI, as shown in Tables (6,7,8,10,11,12,14,15,16,18,19,20), reveals the following:

3.1. Spatial and Temporal Distribution of Contamination

The results revealed significant variation in contamination levels across sites and seasons, with cadmium (Cd) emerging as the most prominent environmental concern. In West Qurna-1, Cd had the highest contamination factor (CF = 10.973) during summer, reflecting intensive industrial activities at this site. Similarly, elevated levels were observed in Seba during winter (CF = 13.3), indicating the long-term accumulation of this toxic metal.

3.2. Anthropogenic vs. Natural Sources

The enrichment factor (EF) values were predominantly anthropogenic sources of pollution, particularly cadmium and lead. Cadmium showed EF values exceeding 40 at most sites, peaking at 121.959 in Al-Zubair during the winter. These high values strongly suggest oil-related industrial waste is the primary source, including incomplete fuel combustion and equipment corrosion [24].

3.3. Seasonal Dynamics and Impacts

- Summer/Autumn: Associated with higher pollution levels due to increased industrial activity and evaporation rates that concentrate metals in the surface layers [25].
- Winter/Spring: Despite some indicator decreases, critical contamination hotspots persisted, such as Seba in winter, indicating chronic metal accumulation [26].

3.4. Comprehensive Pollution Index Assessment

Geoaccumulation Index (I-geo): Confirmed cadmium dominance, with positive values at sites like West Qurna-1 (2.871) and Seba (2.859), while other metals remained at natural background levels.

Pollution Load Index (PLI): Identified priority sites such as West Qurna-1 and Seba requiring urgent intervention, where PLI values exceeded safe thresholds (1.0) across multiple seasons.

Although residual fraction metals are considered less mobile, their persistently high concentrations pose long-term risks, particularly under changing environmental conditions such as pH reduction or soil erosion [27]. The bioaccumulation potential of Cd further amplifies its risk in the food chain.

Table 5. Concentration of Residual Heavy Metals (ug/g) dry weight in Summer Season.

Stations	Zn	Cd	Mn	Pb	Fe	Cu	Cr
West Qurna2	39.288	0.804	184.068	39.288	7086.898	29.794	41.333
Seba	45.231	1.114	383.322	2.584	6773.00	14.850	55.341
North Rumaila	38.474	0.474	129.609	3.568	4851.255	6.336	33.069
Al Zubair	58.917	0.767	100.023	13.916	4388.155	53.486	18.368
Majnoon	40.530	0.575	71.847	3.647	5152.388	6.664	22.623
Safwan	60.694	1.396	330.052	6.561	9198.399	30.500	72.947
South Rumaila	10.765	0.314	89.808	0.480	3372.278	5.272	47.158
Bergezia	28.675	0.339	83.706	5.731	3630.297	8.506	22.028
Shuaaba	36.782	0.304	73.543	5.614	3475.508	14.816	16.046
Ratawi	33.939	0.577	164.053	4.973	5219.880	7.209	34.407
West Qurna1	60.318	1.646	396.628	4.813	9279.445	304.929	76.347

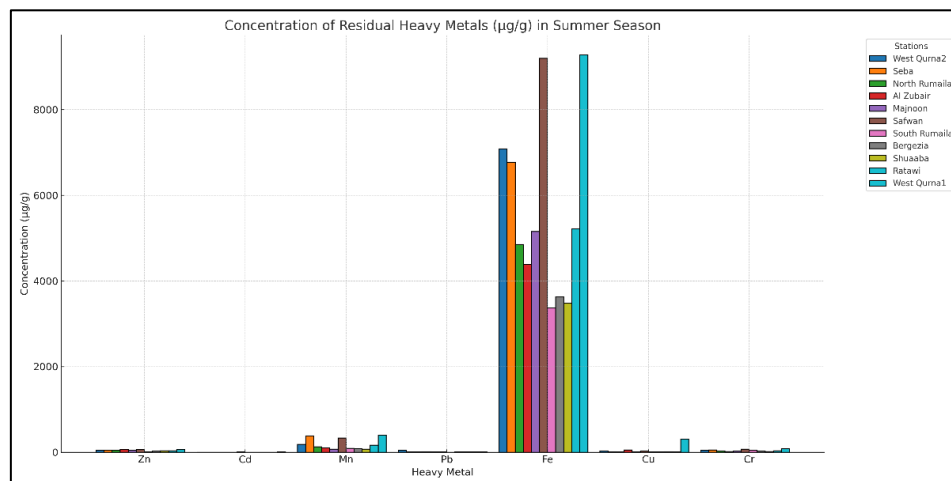


Figure 2. Concentration of Residual Heavy Metals (ug/g) dry weight in Summer Season.

Table 6. Contamination factor (CF) and Pollution Load Index (PLI) of Residual Heavy Metals in Summer Season.

Stations	CF_Zn	CF_Cd	CF_Mn	CF_Pb	CF_Fe	CF_Cu	CF_Cr	PLI
West Qurna2	0.595	5.36	0.174	2.619	0.136	1.145	0.413	0.713
Seba	0.685	7.426	0.362	0.172	0.130	0.571	0.553	0.538
North Rumaila	0.582	3.16	0.122	0.237	0.093	0.243	0.330	0.327
Al Zubair	0.892	5.113	0.094	0.927	0.084	2.057	0.183	0.536
Majnoon	0.614	3.833	0.067	0.243	0.099	0.256	0.226	0.300
Safwan	0.919	9.306	0.312	0.437	0.176	1.173	0.729	0.780
South Rumaila	0.163	2.093	0.084	0.032	0.064	0.202	0.471	0.178
Bergezia	0.434	2.26	0.079	0.382	0.069	0.327	0.220	0.284
Shuaaba	0.557	2.026	0.069	0.374	0.066	0.569	0.160	0.291
Ratawi	0.514	3.846	0.155	0.331	0.100	0.277	0.344	0.371
West Qurna1	0.913	10.973	0.374	0.320	0.178	11.728	0.763	1.098

Table 7. Enrichment Factor (EF) of Residual Heavy Metals in summer season.

Stations	EF_Zn	EF_Cd	EF_Mn	EF_Pb	EF_Cu	EF_Cr
West Qurna2	4.367	39.328	1.276	19.218	8.408	3.032
Seba	5.261	57.018	2.781	1.322	4.385	4.248
North Rumaila	6.248	33.871	1.313	2.549	2.612	3.544
Al Zubair	10.578	60.593	1.120	10.993	24.377	2.176
Majnoon	6.197	38.687	0.685	2.453	2.586	2.283
Safwan	5.198	52.612	1.763	2.472	6.631	4.123
South Rumaila	2.515	32.278	1.308	0.493	3.126	7.271
Bergezia	6.223	32.372	1.133	5.472	4.686	3.155
Shuaaba	8.338	30.322	1.04	5.599	8.525	2.400
Ratawi	5.122	38.320	1.544	3.302	2.762	3.427
West Qurna1	5.121	61.492	2.100	1.798	65.721	4.278

Table 8. The Geochemical Accumulation Coefficient (I-geo) of Residual Heavy Metals in summer season.

Stations	Igeo_Zn	Igeo_Cd	Igeo_Mn	Igeo_Pb	Igeo_Fe	Igeo_Cu	Igeo_Cr
West Qurna2	-1.333	1.837	-3.108	0.804	-0.388	-1.859	-3.460
Seba	-1.130	2.307	-2.049	-3.122	-1.393	-1.438	-3.525
North Rumaila	-1.363	1.075	-3.614	-2.656	-2.621	-2.181	-4.00
Al Zubair	-0.748	1.769	-3.987	-0.693	0.455	-3.029	-4.151
Majnoon	-1.288	1.353	-4.465	-2.625	-2.549	-2.729	-3.920
Safwan	-0.705	2.633	-2.265	-1.777	-0.354	-1.04	-3.084
South Rumaila	-3.201	0.480	-4.143	-5.550	-2.887	-1.669	-4.531
Bergezia	-1.787	0.591	-4.244	-1.973	-2.196	-2.767	-4.425
Shuaaba	-1.428	0.434	-4.431	-2.002	-1.396	-3.224	-4.488
Ratawi	-1.544	1.358	-3.274	-2.177	-2.435	-2.124	-3.901
West Qurna1	-0.714	2.871	-2.000	-2.224	2.966	-0.974	-3.071

Table 9. Concentration of Residual Heavy Metals (ug/g) dry weight in Autumn Season.

Stations	Zn	Cd	Mn	Pb	Fe	Cu	Cr
West Qurna2	65.646	1.815	526.347	5.402	9252.295	33.315	1.993
Seba	51.057	1.111	259.434	8.566	7792.829	26.266	4.244
North Rumaila	57.264	1.372	412.888	4.492	8710.383	31.390	2.8799
Al Zubair	83.217	0.461	137.475	5.532	4925.049	51.940	8.196
Majnoon	51.564	1.055	260.585	4.894	7273.709	47.583	4.245
Safwan	42.855	0.654	171.017	4.381	5387.329	23.335	6.636
South Rumaila	31.258	0.708	248.307	5.897	5059.403	21.209	4.388
Bergezia	18.853	0.505	145.108	4.950	4387.354	48.607	7.512
Shuaaba	47.233	0.533	152.847	10.362	4593.259	36.124	7.848
Ratawi	32.876	1.065	321.859	2.891	7605.167	18.723	3.456
West Qurna1	72.801	1.063	251.928	24.318	6524.381	49.027	4.188

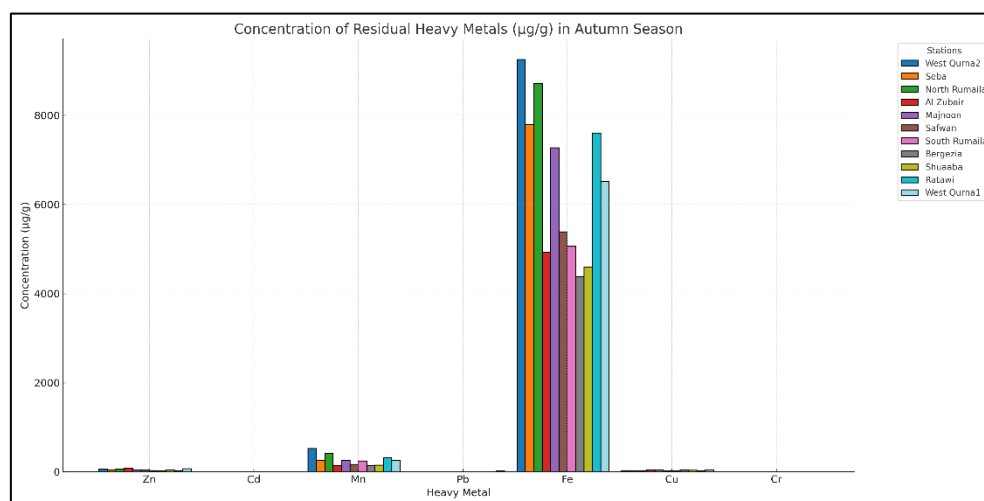


Figure 3. Concentration of Residual Heavy Metals (ug/g) dry weight in Autumn Season.

Table 10. Contamination factor (CF) and Pollution Load Index (PLI) of Residual Heavy Metals in Autumn Season.

Stations	CF_Zn	CF_Cd	CF_Mn	CF_Pb	CF_Fe	CF_Cu	CF_Cr	PLI
West Qurna2	0.994	12.1	0.497	0.360	0.177	1.281	0.019	0.616
Seba	0.773	7.406	0.245	0.571	0.149	1.010	0.042	0.570
North Rumaila	0.867	9.146	0.390	0.299	0.167	1.207	0.028	0.564
Al Zubair	1.260	3.073	0.129	0.368	0.094	1.997	0.082	0.558
Majnoon	0.781	7.033	0.246	0.326	0.139	1.830	0.042	0.57
Safwan	0.649	4.36	0.161	0.292	0.103	0.897	0.066	0.446
South Rumaila	0.473	4.72	0.234	0.393	0.097	0.815	0.043	0.441
Bergezia	0.285	3.366	0.137	0.33	0.084	1.869	0.075	0.427
Shuaaba	0.715	3.553	0.144	0.690	0.088	1.389	0.078	0.55
Ratawi	0.498	7.1	0.304	0.192	0.146	0.720	0.034	0.415
West Qurna1	1.103	7.086	0.238	1.621	0.125	1.885	0.041	0.787

Table 11. Enrichment Factor (EF) of Residual Heavy Metals in Autumn season.

Stations	EF_Zn	EF_Cd	EF_Mn	EF_Pb	EF_Cu	EF_Cr
West Qurna2	5.590	68.004	2.796	2.024	7.201	0.112
Seba	5.162	49.423	1.636	3.810	6.741	0.283
North Rumaila	5.179	54.604	2.329	1.787	7.207	0.171
Al Zubair	13.312	32.449	1.371	3.893	21.092	0.865
Majnoon	5.585	50.281	1.760	2.332	13.083	0.303
Safwan	6.267	42.083	1.560	2.819	8.662	0.640
South Rumaila	4.867	48.511	2.412	4.040	8.384	0.451
Bergezia	3.385	39.902	1.625	3.911	22.157	0.890
Shuaaba	8.101	40.227	1.635	7.820	15.729	0.888
Ratawi	3.405	48.545	2.080	1.317	4.923	0.236
West Qurna1	8.791	56.481	1.897	12.921	15.028	0.333

Table 12. The Geochemical Accumulation Coefficient (I-geo) of Residual Heavy Metals in Autumn season.

Stations	Igeo_Zn	Igeo_Cd	Igeo_Mn	Igeo_Pb	Igeo_Fe	Igeo_Cu	Igeo_Cr
West Qurna2	-0.592	3.012	-1.592	-2.058	-3.075	-0.227	-6.233
Seba	-0.955	2.303	-2.612	-1.393	-3.323	-0.570	-5.143
North Rumaila	-0.789	2.608	-1.942	-2.324	-3.162	-0.313	-5.702
Al Zubair	-0.250	1.034	-3.529	-2.024	-3.985	0.413	-4.193
Majnoon	-0.941	2.229	-2.606	-2.200	-3.422	0.287	-5.143
Safwan	-1.208	1.539	-3.214	-2.360	-3.855	-0.741	-4.498
South Rumaila	-1.663	1.653	-2.676	-1.931	-3.946	-0.878	-5.095
Bergezia	-2.392	1.166	-3.451	-2.184	-4.152	0.317	-4.319
Shuaaba	-1.067	1.244	-3.376	-1.118	-4.085	-0.110	-4.256
Ratawi	-1.590	2.242	-2.301	-2.960	-3.358	-1.058	-5.439
West Qurna1	-0.443	2.240	-2.655	0.112	-3.579	0.330	-5.162

Table 13. Concentration of Residual Heavy Metals (ug/g) dry weight in Winter Season.

Stations	Zn	Cd	Mn	Pb	Fe	Cu	Cr
West Qurna2	51.284	1.327	295.752	13.130	7699.839	29.766	55.597
Seba	78.745	1.995	313.776	17.852	8075.076	25.519	68.585
North Rumaila	61.890	0.712	84.091	8.255	3279.458	15.528	18.448
Al Zubair	52.821	1.142	74.607	15.272	3246.11	18.968	16.148
Majnoon	51.372	0.923	225.890	8.8190	7046.416	22.608	41.750
Safwan	37.351	0.926	215.044	9.3152	6603.708	14.514	41.391
South Rumaila	63.548	1.434	153.456	15.905	5893.078	19.057	33.319
Bergezia	82.719	1.482	224.837	11.822	8433.112	30.355	69.411
Shuaaba	42.908	1.251	142.220	14.872	5249.087	18.465	27.780
Ratawi	67.931	1.630	337.492	8.698	8851.852	24.378	89.351
West Qurna1	55.871	1.766	295.878	12.099	7894.245	18.710	57.398

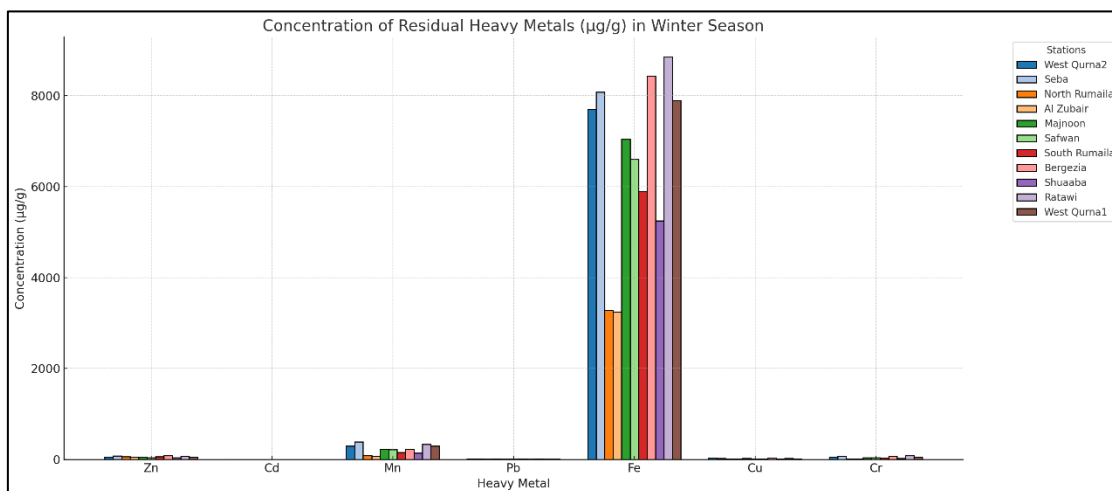


Figure 4. Concentration of Residual Heavy Metals (ug/g) dry weight in Winter Season.

Table 14. Contamination factor (CF) and Pollution Load Index (PLI) of Residual Heavy Metals in Winter Season

Stations	CF_Zn	CF_Cd	CF_Mn	CF_Pb	CF_Fe	CF_Cu	CF_Cr	PLI
West Qurna2	0.777	8.846	0.279	0.875	0.148	1.144	0.556	1.011
Seba	1.193	13.3	0.296	1.190	0.155	0.981	0.685	1.247
North Rumaila	0.937	4.746	0.079	0.550	0.063	0.597	0.184	0.527
Al Zubair	0.800	7.613	0.070	1.018	0.062	0.729	0.161	0.61
Majnoon	0.778	6.153	0.213	0.587	0.135	0.869	0.417	0.775
Safwan	0.565	6.173	0.203	0.621	0.127	0.558	0.413	0.683
South Rumaila	0.962	9.56	0.145	1.060	0.113	0.733	0.333	0.837
Bergezia	1.253	9.88	0.212	0.788	0.162	1.167	0.694	1.090
Shuaaba	0.650	8.34	0.134	0.991	0.100	0.710	0.277	0.722
Ratawi	1.029	10.866	0.319	0.579	0.170	0.937	0.893	1.096
West Qurna1	0.846	11.773	0.279	0.806	0.151	0.719	0.574	0.987

Table 15. Enrichment Factor (EF) of Residual Heavy Metals in Winter season.

Stations	EF_Zn	EF_Cd	EF_Mn	EF_Pb	EF_Cu	EF_Cr
West Qurna2	5.247	59.745	1.887	5.911	7.731	3.754
Seba	7.683	85.646	1.909	7.663	6.320	4.416
North Rumaila	14.868	75.264	1.260	8.726	9.469	2.925
Al Zubair	12.820	121.959	1.129	16.309	11.686	2.586
Majnoon	5.744	45.409	1.575	4.338	6.416	3.081
Safwan	4.456	48.611	1.600	4.890	4.395	3.259
South Rumaila	8.496	84.356	1.279	9.356	6.467	2.94
Bergezia	7.728	60.921	1.310	4.859	7.199	4.28
Shuaaba	6.440	82.620	1.331	9.821	7.035	2.752
Ratawi	6.046	63.836	1.873	3.406	5.508	5.248
West Qurna1	5.576	77.551	1.842	5.313	4.740	3.780

Table 16. The Geochemical Accumulation Coefficient (I-geo) of Residual Heavy Metals in Winter season.

Stations	Igeo_Zn	Igeo_Cd	Igeo_Mn	Igeo_Pb	Igeo_Fe	Igeo_Cu	Igeo_Cr
West Qurna2	-0.948	2.560	-2.423	-0.777	-3.340	-0.389	-1.431
Seba	-0.330	3.148	-2.338	-0.333	-3.271	-0.611	-1.129
North Rumaila	-0.677	1.662	-4.238	-1.446	-4.571	-1.328	-3.023
Al Zubair	-0.906	2.343	-4.410	-0.559	-4.586	-1.039	-3.215
Majnoon	-0.946	2.036	-2.812	-1.351	-3.468	-0.786	-1.845
Safwan	-1.406	2.041	-2.883	-1.272	-3.562	-1.426	-1.857
South Rumaila	-0.639	2.672	-3.370	-0.500	-3.726	-1.033	-2.170
Bergezia	-0.259	2.719	-2.819	-0.928	-3.209	-0.361	-1.111
Shuaaba	-1.206	2.475	-3.480	-0.597	-3.893	-1.078	-2.432
Ratawi	-0.543	2.856	-2.233	-1.371	-3.139	-0.677	-0.747
West Qurna1	-0.825	2.972	-2.423	-0.895	-3.304	-1.059	-1.385

Table 17. Concentration of Residual Heavy Metals (ug/g) dry weight in Spring Season.

Stations	Zn	Cd	Mn	Pb	Fe	Cu	Cr
West Qurna2	0.340	0.003	0.404	0.074	31.355	0.105	0.444
Seba	58.462	1.633	386.495	17.769	9676.116	22.446	83.490
North Rumaila	0.352	0.006	0.630	0.096	55.901	0.136	0.874
Al Zubair	0.269	0.001	0.288	0.052	19.713	0.050	0.410
Majnoon	49.095	0.853	164.346	14.216	7097.361	16.771	36.877
Safwan	26.368	0.320	77.458	14.146	2894.861	10.005	15.710
South Rumaila	0.380	0.005	0.616	0.144	38.994	0.1764	0.879
Bergezia	0.009	0.001	0.008	0.022	1.263	0.001	0.101
Shuaaba	0.511	0.008	0.910	0.366	51.51	0.204	1.381
Ratawi	0.144	0.004	0.179	0.067	71.603	0.027	0.386
West Qurna1	0.356	0.005	0.645	0.050	36.043	0.185	0.881

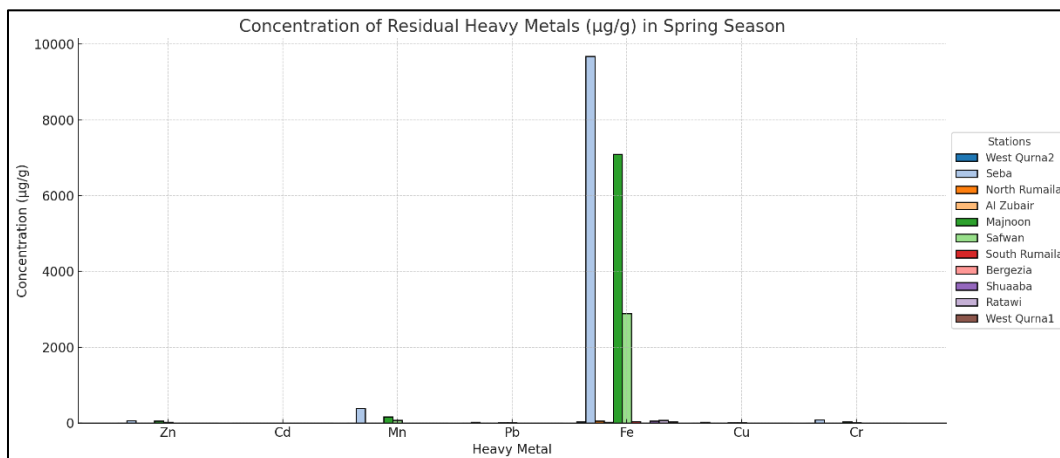


Figure 5. Concentration of Residual Heavy Metals ($\mu\text{g/g}$) dry weight in Spring Season.

Table 18. Contamination factor (CF) and Pollution Load Index (PLI) of Residual Heavy Metals in Spring Season.

Stations	CF_Zn	CF_Cd	CF_Mn	CF_Pb	CF_Fe	CF_Cu	CF_Cr	PLI
West Qurna2	0.005	0.02	0.003	0.004	0.006	0.004	0.004	0.003
Seba	0.885	10.886	0.365	1.184	0.186	0.863	0.834	1.201
North Rumaila	0.005	0.04	0.005	0.006	0.001	0.005	0.008	0.005
Al Zubair	0.004	0.006	0.002	0.0034	0.003	0.001	0.004	0.002
Majnoon	0.743	5.686	0.155	0.947	0.136	0.645	0.368	0.727
Safwan	0.399	2.133	0.073	0.943	0.055	0.384	0.157	0.390
South Rumaila	0.005	0.033	0.005	0.009	0.007	0.0067	0.008	0.006
Bergezia	0.001	0.006	7.56	0.0014	2.43	3.85	0.001	0.002
Shuaaba	0.007	0.053	0.008	0.024	0.009	0.007	0.013	0.009
Ratawi	0.002	0.026667	0.001	0.004	0.001	0.001	0.003	0.002
West Qurna1	0.005	0.033333	0.006	0.003	0.006	0.007	0.008	0.005

Table 19. Enrichment Factor (EF) of Residual Heavy Metals in Spring season.

Stations	EF_Zn	EF_Cd	EF_Mn	EF_Pb	EF_Cu	EF_Cr
West Qurna2	8.543	33.168	0.633	8.181	6.697	7.363
Seba	4.760	58.505	1.963	6.366	4.639	4.486
North Rumaila	4.961	37.208	0.553	5.953	4.865	8.130
Al Zubair	10.751	17.585	0.718	9.144	5.072	10.815
Majnoon	5.450	41.664	1.138	6.943	4.725	2.701
Safwan	7.176	38.320	1.315	16.940	6.912	2.821
South Rumaila	7.677	44.451	0.776	12.801	9.047	11.721
Bergezia	5.614	274.478	0.311	60.385	1.583	41.583
Shuaaba	7.816	53.840	0.868	24.632	7.920	13.941
Ratawi	1.584	19.366	0.122	3.243	0.754	2.803
West Qurna1	7.781	48.090	0.879	4.809	10.265	12.710

Table 20. The Geochemical Accumulation Coefficient (I-geo) of Residual Heavy Metals in Spring season.

Stations	Igeo_Zn	Igeo_Cd	Igeo_Mn	Igeo_Pb	Igeo_Fe	Igeo_Cu	Igeo_Cr
West Qurna2	-8.185	-6.228	-11.939	-8.248	-11.280	-8.536	-8.400
Seba	-0.759	2.859	-2.037	-0.340	-3.010	-0.797	-0.845
North Rumaila	-8.135	-5.228	-11.298	-7.872	-10.446	-8.163	-7.423
Al Zubair	-8.523	-7.813	-12.427	-8.757	-11.950	-9.607	-8.515
Majnoon	-1.011	1.922	-3.271	-0.662	-3.458	-1.217	-2.024
Safwan	-1.908	0.508	-4.356	-0.669	-4.751	-1.962	-3.255
South Rumaila	-8.025	-5.491	-11.331	-7.287	-10.966	-7.788	-7.414
Bergezia	-13.425	-7.813	-17.597	-9.998	-15.914	-15.251	-10.536
Shuaaba	-7.597	-4.813	-10.768	-5.941	-10.564	-7.578	-6.763
Ratawi	-9.425	-5.813	-13.114	-8.391	-10.089	-10.496	-8.602
West Qurna1	-8.119	-5.491	-11.264	-8.813	-11.079	-7.719	-7.411

3.5. Statistical Analysis and Interpretation of Seasonal and Spatial Variations in Heavy Metal Concentrations

The Kruskal–Wallis test results showed significant seasonal differences in soil heavy metal concentrations, but insignificant spatial variation. Seasonal effects—specifically cycles of wetting/drying and temperature-induced changes in soil chemistry—are key drivers of heavy metal mobility, as demonstrated by [28,29]. This highlights that environmental conditions over time are more influential than geographic location in shaping metal distribution.

The homogeneity across sampling sites suggests widespread pollution sources, likely linked to regional oil industry emissions rather than discrete point sources. Comparable patterns have been observed in conflict-affected regions of Iraq, where contamination appeared uniformly distributed across sampling zones [30,31].

Table 21. Results of Kruskal–Wallis Test for Seasonal and Spatial Variations in Residual Heavy Metal Concentrations in Soil Samples.

Metal	Season KW statistic	p-value (seasons)	Station KW statistic	p-value (sites)
Zn	18.756	< 0.001	6.312	0.788 (n.s.)
Cd	19.204	< 0.001	9.054	0.527 (n.s.)
Mn	15.282	0.002	13.206	0.212 (n.s.)
Pb	13.874	0.003	5.442	0.860 (n.s.)
Fe	12.806	0.005	13.273	0.209 (n.s.)
Cu	22.710	< 0.001	4.958	0.894 (n.s.)
Cr	24.904	< 0.001	5.830	0.829 (n.s.)

n.s. = not significant ($p > 0.05$)

As illustrated in Table (22), the comparison demonstrates considerable discrepancies in heavy metal contamination levels between the present study and earlier investigations conducted in the oil-producing areas of Basra. This highlights the pressing need for enhanced regulation of industrial emissions and the implementation of long-term environmental monitoring programs. The findings emphasize the critical role of conducting regular assessments to evaluate the effectiveness of environmental protection strategies, particularly in ecologically sensitive and industrially active zones.

Table 22. Heavy metals concentrations ($\mu\text{g/g}$ dry weight) in the present study as compared with the other previous studies.

Study area	Zn	Cd	Mn	Pb	Fe	Cu	Cr	References
Khor Al-Zubair	27.41-58.48	-	353.7-570.6	-	6676.0-7398.3	7.56-27.73	-	[32]
Qurna-2 oil field	25.23-40.48	5.99-10.23	-	- 18.23-39.15	1150.12-2185.83	10.12-18.22	50.64-59.23	[33]
Qurna-1 oil field	-	9.79-19.65	91.65-111.5	-	-	6.18-14.42	-	[34]
Rumaila oil field	10.69	0.92	-	17.17	1511.17	39.24	-	[35]
Basrah Oil Fields	0.009-83.217	0.001-1.995	0.008-526.347	0.022-39.288	1.236-9676.116	0.001-304.929	0.101-89.351	Present study

Conclusions

The seasonal analysis of residual heavy metals in southern Iraq revealed that autumn and winter recorded the highest concentrations due to industrial emissions and atmospheric deposition, while spring showed the lowest levels, likely due to dilution. Cadmium (Cd) showed the most critical contamination indicators (CF, EF, Igeo), particularly in West Qurna-1, Seba, and Al-Zubair, linking it strongly to oil-related activities. Despite the residual phase being less bioavailable, elevated levels of Cd and Pb pose long-term ecological risks. The Pollution Load Index (PLI) identified West Qurna-1 and Seba as critical hotspots, emphasizing the need for immediate environmental management. These findings stress the importance of considering seasonal variation in monitoring programs and addressing pollution sources to protect soil health and food safety.

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The Effect of Jasmonic Acid and Growth Regulators on the Production of Compounds from Tissue Cultures of Date Palm (*Phoenix dactylifera* L. cv. Barhi) with the Possibility of Using it as an Inhibitor of Some Bacteria

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Abstract. This study aimed to evaluate the biological activity of alcoholic extracts obtained from tissue cultures of the date palm (*Phoenix dactylifera* L. cv. Barhi) under the influence of growth regulators and jasmonic acid. The results showed that the various treatments had a clear impact on the production of chemical compounds in terms of both quantity and type, and also revealed variability in inhibitory activity against bacteria. Treatment B, which involved a moderate concentration of jasmonic acid and growth regulators, recorded the highest number of compounds (50 compounds) and demonstrated superior biological activity compared to other treatments, with inhibition zone diameters reaching 30 mm and 29 mm against *E. coli* and *S. aureus*, respectively, at the highest concentration. Treatments D, E, and F exhibited moderate activity, whereas treatment A exhibited the lowest effectiveness. These findings indicate that jasmonic acid, in interaction with growth regulators, contributes to the stimulation of biologically active compound production, most notably phenolics and flavonoids, which play a significant role in antibacterial activity. These extracts are promising natural sources of bioactive agents, paving the way for their potential use in pharmaceutical and preventive applications.

Keywords. Callus culture, GC-MS analysis, Bioactive metabolites, Antibacterial inhibition zones, Phenolic compounds, In vitro elicitation.

1. Introduction

Since the dawn of history, humans have relied on plants as an essential source of food and medicine. Over the generations, knowledge of medicinal plants has evolved through trial and error. The therapeutic effects of plants are attributed to the presence of complex chemical compounds known as secondary metabolites, which exhibit antimicrobial properties and are beneficial for treating various diseases [1-3].

The date palm (*Phoenix dactylifera* L.) is one of the most economically important crops in the Middle East and belongs to the family Arecaceae. Date fruits are well known for their high nutritional value, and traditionally, all parts of the palm tree are utilized in daily life [4,5]. Date palm is considered a rich source of essential minerals, with potassium, magnesium, and sodium being the most abundant elements found in the fruit [6,7]. In addition, *P. dactylifera* pits are recognized as a valuable source of healthy oils, particularly oleic acid, which can be utilized in the production of pharmaceutical ingredients [8]. Due to the presence of bioactive compounds, dates exhibit significant free radical scavenging capacity [7,9]. They also demonstrate antimicrobial activity [10], and possess anticancer properties [8]. Furthermore, most date-based products display notable antioxidant and antimutagenic effects [11]. The health benefits of dates are primarily attributed to their rich content of phytochemicals, which are known for their pharmacological properties. These include flavonoids, carotenoids, phenolic acids, sterols, procyanidins, and anthocyanins. In recent years, in vitro cell culture technology has emerged as a promising tool for the production of biomass and bioactive compounds [12].

Due to the limitations of traditional propagation methods, mainly the low number of offshoots and the long time required for root development, alternative techniques, such as plant tissue culture, have become necessary. Tissue culture offers disease-free, genetically uniform plants and enables year-round mass production [13-15].

Plant tissue culture has proven efficient in producing medically important phytochemicals such as phenolics, alkaloids, and flavonoids, which are often difficult or impossible to synthesize chemically, making tissue-derived sources a practical alternative [16,17]. The success of in vitro propagation largely depends on the composition of the culture medium and the suitability of the added plant growth regulators, particularly auxins and cytokinins, which influence cell division and differentiation. These regulators also interact with other hormones such as jasmonates (JAs), which are potent inducers of secondary metabolite biosynthesis [18,19].

Jasmonic acid plays a key role as an elicitor of bioactive compound production in plant. It is used in tissue culture systems to stimulate callus formation and synthesis of defensive compounds, such as phenolics and flavonoids. Moreover, it activates secondary metabolic pathways in response to environmental or chemical stress [20-22].

However, assessing the biological activity of extracted compounds through microbial inhibition is a critical step in evaluating their effectiveness. *Staphylococcus aureus* and *Escherichia coli* are commonly used model organisms in microbiological studies because of their clinical significance and increasing antibiotic resistance [23,24].

2. Materials and Methods

2.1. Sample Collection and Tissue Preparation

The shoots of *Phoenix dactylifera* L. cv. Barhi, aged between 3 and 4 years and free from diseases, was collected from a local orchard in Najaf Governorate in October 2024. Offshoots were transported to the Tissue Culture Laboratory at the Directorate of Agriculture in Al-Najaf Al-Ashraf. Shoot apical meristem tissues were excised and washed under running tap water, followed by several rinses with distilled water. The tissues then underwent a meticulous sterilization process according to the standard protocols adopted in the laboratory, in preparation for culture using tissue culture techniques.

Bacterial isolates under investigation, two clinically common pathogenic species, *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative), were also collected. The isolates were obtained from local clinical sources and cultured on appropriate nutrient medium. They

were reactivated in a microbiology laboratory to ensure their viability and readiness for use in biological inhibition assays [25].

2.2. Preparation of Culture Medium (MS)

Plant culture medium was prepared using the Murashige and Skoog [26] formulation at a concentration of 4.44 g/L, supplemented with sucrose at 30 g/L. The final volume was adjusted to one liter using distilled water. The pH was adjusted to 5.7 using either NaOH or HCl. The agar was added at a concentration of 8 g/L to solidify the medium. The mixture was then heated using a magnetic stirrer until all components were completely dissolved, and subsequently dispensed into glass tubes at 10 mL per tube.

2.3. Hormonal Treatments and Sterilization

A series of hormonal treatments was prepared to evaluate the interactive effect between growth regulators and jasmonic acid in stimulating the production of bioactive compounds. These treatments included three concentrations of jasmonic acid (0, 2, and 4 mg/l), in addition to different growth regulators: 2iP at 2.4 mg/l, 2,4-D at two concentrations (8 and 12 mg/l), and NAA at two concentrations (40 and 60 mg/l). The treatments were organized into six main experimental groups (A–F), as detailed in Table (1).

Table 1. Concentrations of jasmonic acid and other growth regulators added to the MS culture medium.

Treatment	JA (mg/l)	2iP (mg/l)	2,4-D (mg/l)	NAA (mg/l)
A (control)	0	2	8	40
B	2	2	8	40
C	4	2	8	40
D	0	4	12	60
E	2	4	12	60
F	4	4	12	60

After preparing the culture medium containing the respective growth regulators and jasmonic acid according to each treatment, it was sterilized using an autoclave at 121°C and a pressure of 1.04 kg/cm² for 21 minutes. The medium was then allowed to cool to room temperature and was ready for use in tissue culture [27].

2.4. Callus Induction in the Growth Chamber

Following sterilization, the shoot apices were cultured in glass tubes containing 10 mL of pre-prepared culture medium supplemented with growth regulators and jasmonic acid according to each of the six treatments (A–F). A shoot apex was placed in each tube.

After inoculation, the tubes were transferred to a plant tissue culture growth chamber under controlled environmental conditions. The temperature was maintained at 25±2°C with a photoperiod of 16 h of light, followed by 8 h of darkness. Illumination was provided by 40-watt white fluorescent tubes arranged to ensure uniform light distribution throughout the chamber (Figure 1).



Figure 1. Tissue culture growth room during date palm callus induction.

The cultures were incubated for eight weeks, during which morphological changes in the shoot apices were monitored. Tissue swelling was observed beginning in the second week, followed by the formation of an initial callus at the base of the apices in the subsequent weeks (Figure 2) [28]. At the end of the designated culture period, the induced calli were carefully collected and stored under the appropriate conditions for subsequent drying, alcoholic extraction, and GC-MS analysis.

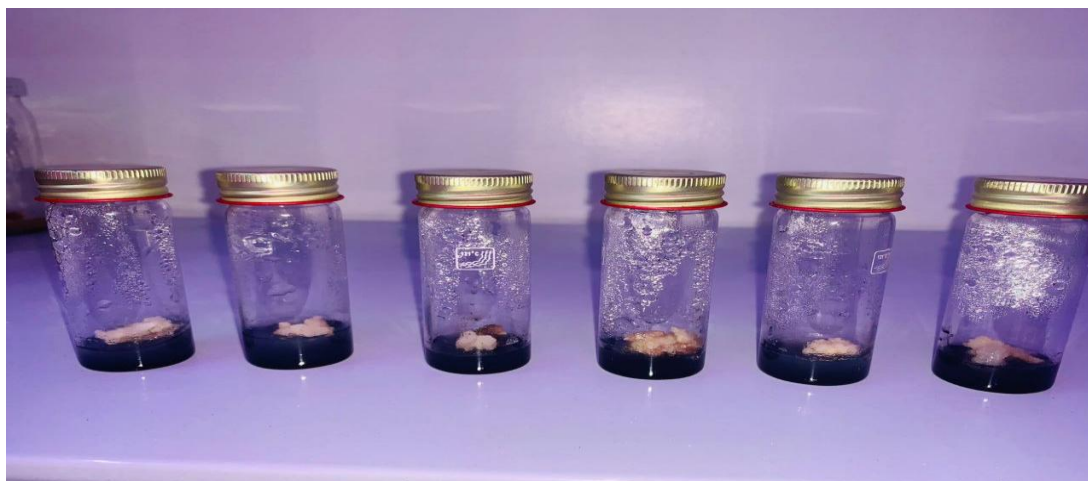


Figure 2. Callus formed from shoot apices of *Phoenix dactylifera* L. cv. Barhi after incubation in glass tubes.

2.5. Compound Extraction and GC-MS Analysis

Callus tissues obtained from the different treatments were dried in an oven at an appropriate temperature and then ground into a fine powder. A specific quantity of this powdered material was subjected to alcoholic extraction using 70% ethanol, following the standard extraction ratio established in the laboratory. Upon completion of the extraction process, the extracts were filtered using filter paper(Whatman No.1), and the filtrate was collected for further analysis.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed to identify the chemical compounds present in alcoholic extracts. The analysis was conducted at the laboratories of the College of Science, University of Al-Qadisiyah, using a Shimadzu GC-MS system (Japan).

The obtained compound spectra were compared with the NIST compound database to identify the components of the extract.

2.6. Statistical Analysis

The experimental results were analyzed using a Completely Randomized Design (CRD) to evaluate the differences among treatments. Mean comparisons were performed using the Least Significant Difference (LSD) test at a significance level of 0.05 to determine the presence of statistically significant differences among the data derived from various treatments. Statistical analysis was conducted using the SPSS software.

3. Results

3.1. Analysis of the Number of Chemical Compounds in the Different Treatments

GC-MS analysis of alcoholic extracts obtained from the callus of *Phoenix dactylifera* L. cv. Barhi, derived from six different treatments (A–F), revealed clear variation in the number of detected chemical compounds. A total of 138 compounds were identified. Treatment A contained the lowest number of compounds (32), followed by treatment C (35). Treatments D, E, and F each yielded an equal number of compounds, totaling 40 per treatment. The highest number was recorded in treatment B, with 50 compounds (Table 2).

The results also revealed the presence of 35 compounds shared among the treatments (Table 3), including 8 compounds that were common to all six treatments. This indicated the biochemical stability of these compounds under various treatment conditions. These differences were statistically significant at a probability level of $P \leq 0.05$, according to the Least Significant Difference (LSD) test.

Table 2. Represents the number of chemical compounds in the Barhi palm callus extract for all treatments.

Sample	Number of chemical compounds
A(control)	32
B	50
C	35
D	40
E	40
F	40

Table 3. Shows some common chemical compounds of Barhi palm callus extract for all treatments.

No.	Compound Name	A	B	C	D	E	F
1	1,2,3-Propanetriol, 1-acetate	5.98	7.52	2.12	9.55	9.02	2.07
2	2-(Isobutoxymethyl)oxirane	8.24	4.87	31.25	8.22	14.62	32.03
3	2,2'-Bioxirane	1.02	1.41	0.44	0.98	0.89	0.57
4	2-Propanone, 1-hydroxy-	1.95	3.02	0.99	1.86	2.24	0.94
5	Benzophenone	2.11	1.75	1.31	1.62	1.58	1.07
6	Dichloroxylenol	3.81	2.72	1.45	1.97	1.48	1.1
7	Glyceraldehyde	1.4	5.59	6.09	0.75	2.54	0.09
8	Propanoic acid, 2-oxo-, methyl ester	1.05	2.47	0.43	0.5	0.5	0.25
9	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	5.83	6.05	0.38	8.25	7.95	
10	Dihydroxyacetone		5.63	8.59	1.12	4.62	7.22

No.	Compound Name	A	B	C	D	E	F
11	Glycerin		0.82	12.81	2.94	2.56	14.93
12	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	1.15	1.01		1.27	1.44	
13	2-Furanmethanol		0.25		0.34	0.36	0.16
14	Butyrolactone	3	1.35		2.3	1.1	
15	Diethoxymethyl acetate	0.93	0.4	0.24		0.42	
16	Ethyl .alpha.-d-glucopyranoside	5.01	14.39		17.72	11.91	
17	Heptanoic acid, 6-oxo-			2.8	8.25	6.27	2.98
18	Methylpyruvate		0.71	0.6		0.75	0.73
19	n-Hexadecanoic acid	1.69	0.76		1.32		0.19
20	Pentanoic acid, 4-oxo-	0.48	0.43		0.65	0.43	
21	1-Hexadecanol	0.68	0.58	3.83			
22	2(3H)-Furanone, dihydro-4-hydroxy-		0.5			0.69	0.66
23	5-Hydroxymethylfurfural			4.68		0.74	2.65
24	Cyclopentane, 1-acetyl-1,2-epoxy-	1.93		2.29		1.31	
25	(S)-(-)-1,2,4-Butanetriol, 2-acetate				3.16	3.29	
26	.alpha.-Terpineol		0.32	0.14			
27	.beta.-D-Glucopyranose, 1,6-anhydro-					2.12	2.12
28	1,3-Methylene-d-arabitol		0.49		3.82		
29	3-Deoxy-d-mannonic lactone			6.15			6.94
30	3-Deoxy-d-mannonic acid			4.8			4.49
31	6-(Hydroxymethyl)-2,4-dihydro-1,4-benzoxazin-3-one				0.51	0.33	
32	Cyclopentanone, 2-methyl-					0.35	0.29
33	Propanoic acid, 3-(acetylthio)-2-methyl-	20.57	8.54				
34	Pyrimidine, 1,4,5,6-tetrahydro-1,2-dimethyl-				0.17		0.43
35	Triethyl phosphate		0.17		0.3		

3.2. Effect of Growth Regulators and Jasmonic Acid on the Increase in the Number of Chemical Compounds

GC-MS analysis results indicated that the interaction between growth regulators and jasmonic acid led to significant differences in the percentage of stable compounds within the total relative areas of the chemical constituents in the alcoholic extracts derived from the callus of *Phoenix dactylifera* L. cv. Barhi. The experimental treatments included the application of growth regulators: 2iP at 2 mg/l, 2,4-D at 8 and 12 mg/l, and NAA at 40 and 60 mg/l, along with jasmonic acid applied at concentrations of 0, 2, and 4 mg/l.

Treatment B, which combined 2 mg/l of 2iP, 8 mg/l of 2,4-D, 40 mg/l of NAA, and 2 mg/l of jasmonic acid, recorded the highest percentage of stability at 59.2% (Table 3), with statistically significant differences at a probability level of $P \leq 0.05$, compared to the other treatments. In contrast, treatment A recorded the lowest percentage at 7.66%, whereas treatments C, D, E, and F had equal percentages of 8.29%.

Chemical analysis also revealed diverse compound classes identified in the extracts, including phenolics, terpenoids, alkaloids, alcohols, ethers, esters, ketones, aldehydes, organic acids, fatty acids, and cyclic hydrocarbons.

Table 4. Represents the percentage of Barhi palm callus extract for all treatments.

Sample	Percentage (%)
A(controh)	7.66
B	59.2
C	8.29
D	8.29

Sample	Percentage (%)
E	8.29
F	8.29

3.3. Biological Activity of Alcoholic Extracts Against Bacteria:

The results of the biological activity assay of the alcoholic extracts derived from the callus of *Phoenix dactylifera* L. cv. Barhi against *Staphylococcus aureus* and *Escherichia coli* revealed clear differences in inhibitory activity among the various treatments, as shown in Table (4). For comparison, the antibiotic disc amoxicillin (AMC) at a concentration of 30 µg/disc was used as a positive control, showing inhibition zone diameters of 17 mm against *E. coli* and 15 mm against *S. aureus*, as illustrated in figure (3). In contrast, treatment B exhibited the highest biological activity, with inhibition zones of 30 mm against *E. coli* and 29 mm against *S. aureus* at a concentration of 300 mg/mL, significantly ($P \leq 0.05$) surpassing all other treatments figure (4). Treatment F exhibited moderate activity, with inhibition zones of 17 and 16 mm against *E. coli* and *S. aureus*, as shown in Figure (9). Treatment C resulted in inhibition zones of 20 mm against *S. aureus* and 16 mm against *E. coli*, indicating moderate effectiveness relative to treatment B (Figure 6). Treatments D and E showed comparable antibacterial activities. Treatment D resulted in an inhibition zone of 21 mm against *S. aureus* and 18 mm against *E. coli* (Figure 7), while treatment E resulted in inhibition zones of 18 mm against both bacterial strains (Figure 8). In contrast, treatment A showed the lowest biological activity, with inhibition zones of 15 mm against *E. coli* and 14 mm against *S. aureus* at 300 mg/mL (Figure 4-8).

These results demonstrate significant differences among the treatments, reflecting the effect of the interaction between growth regulators and jasmonic acid in stimulating the production of highly bioactive compounds, particularly in treatment B, which exhibited the strongest inhibitory activity against both tested bacterial strains.

Table 4. Shows the diameters of the inhibition zones (in mm) resulting from the effect of different plant extracts compared to the antibiotic Amoxyclav against *E. coli* and *S. aureus* at different concentrations.

Sample	Concentration (mg/l)			Bacteria
	(1) 300	(2) 200	(3) 100	
A	15	10	0	<i>E.coli</i>
	14	9	0	<i>S.aures</i>
B	30	20	18	<i>E.coli</i>
	29	20	16	<i>S.aures</i>
C	16	10	8	<i>E.coli</i>
	20	16	13	<i>S.aures</i>
D	16	10	1	<i>E.coli</i>
	14	13	1	<i>S.aures</i>
E	14	12	10	<i>E.coli</i>
	13	12	9	<i>S.aures</i>
F	17	15	14	<i>E.coli</i>
	16	13	12	<i>S.aures</i>
(At a concentration of 30 µg/disc)				
AMC		17		<i>E.coli</i>
		15		<i>S.aures</i>



Figure 3. Antibacterial activity of AMC disc (at a concentration of 30 µg/disc) against *E. coli* and *S. aureus*.

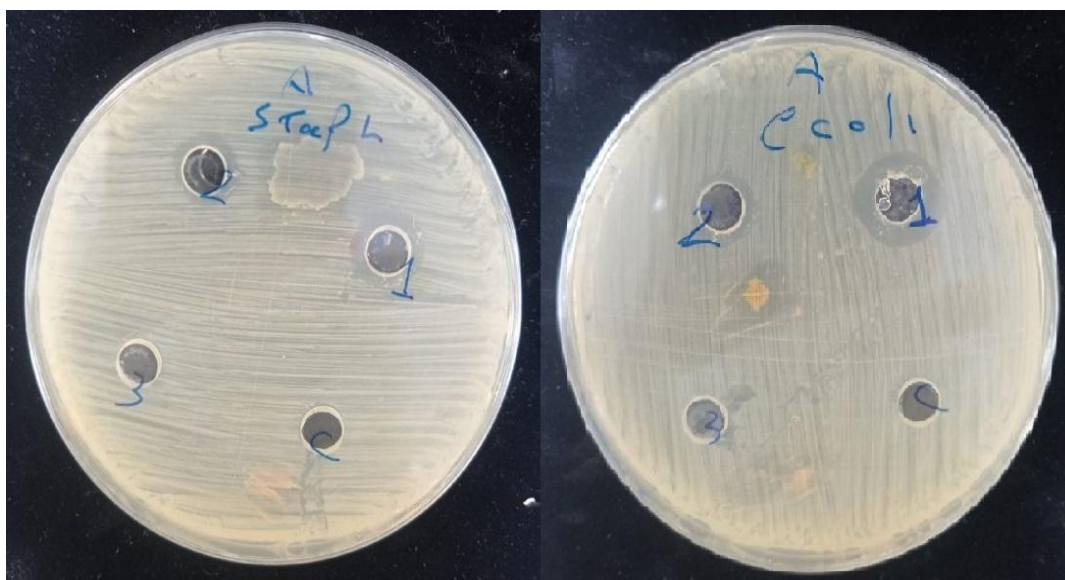


Figure 4. Antibacterial activity of the plant extract from sample A at three concentrations (100, 200, and 300 mg/mL) against *E. coli* and *S. aureus*, with 1% DMSO used as a negative control.

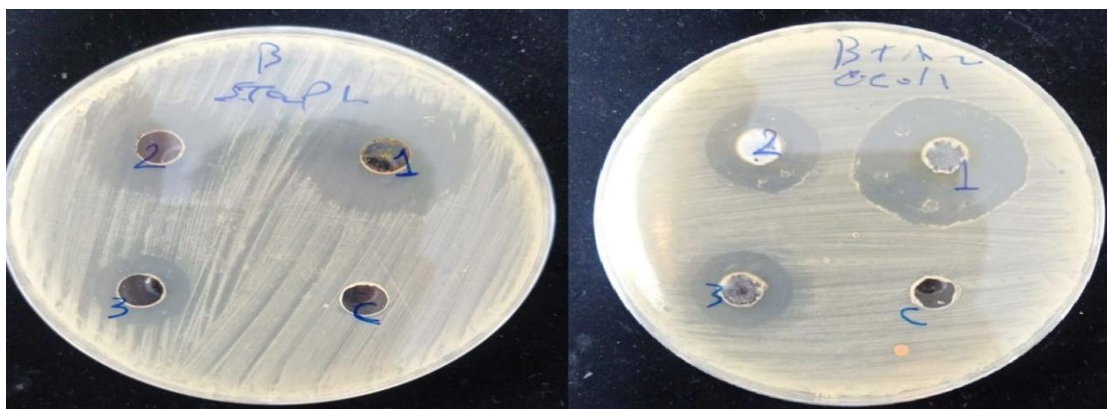


Figure 5. Antibacterial activity of the plant extract from sample B at three concentrations (100, 200, and 300 mg/mL) against *E. coli* and *S. aureus*, with 1% DMSO used as a negative control.

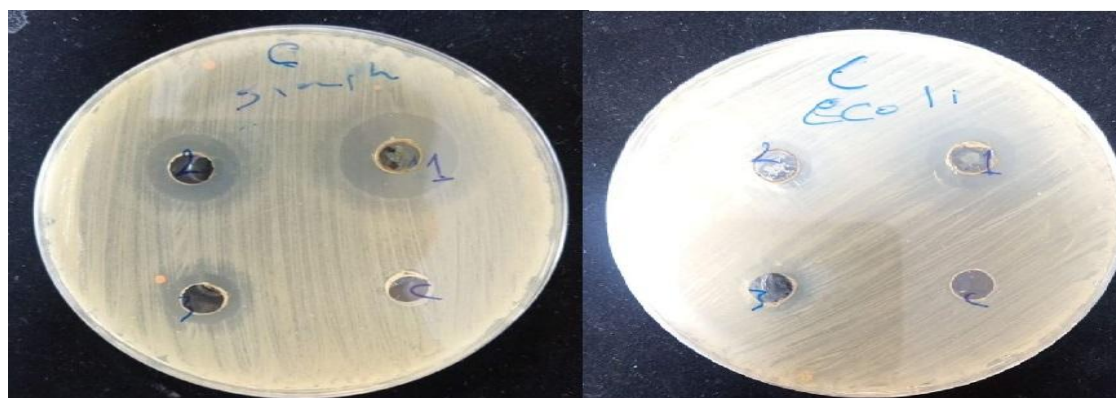


Figure 6. Antibacterial activity of the plant extract from sample C at three concentrations (100, 200, and 300 mg/mL) against *E. coli* and *S. aureus*, with 1% DMSO used as a negative control.

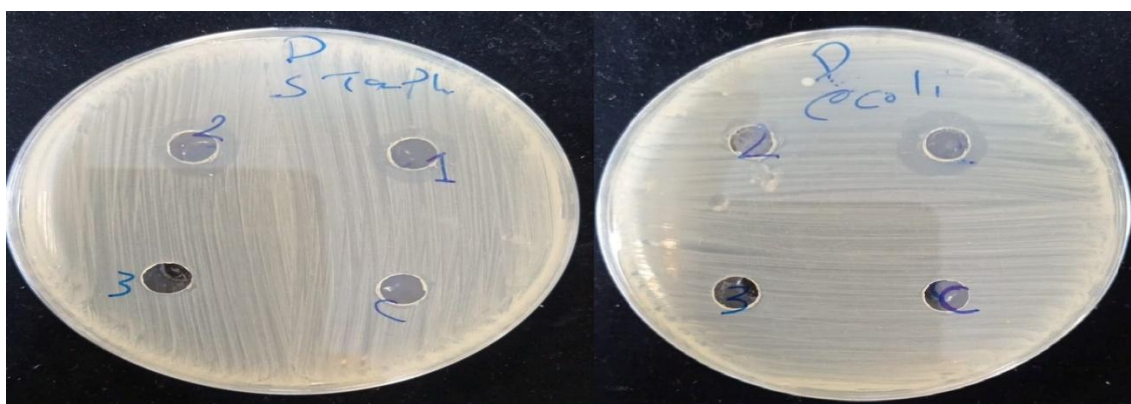


Figure 7. Antibacterial activity of the plant extract from sample D at three concentrations (100, 200, and 300 mg/mL) against *E. coli* and *S. aureus*, with 1% DMSO used as a negative control.

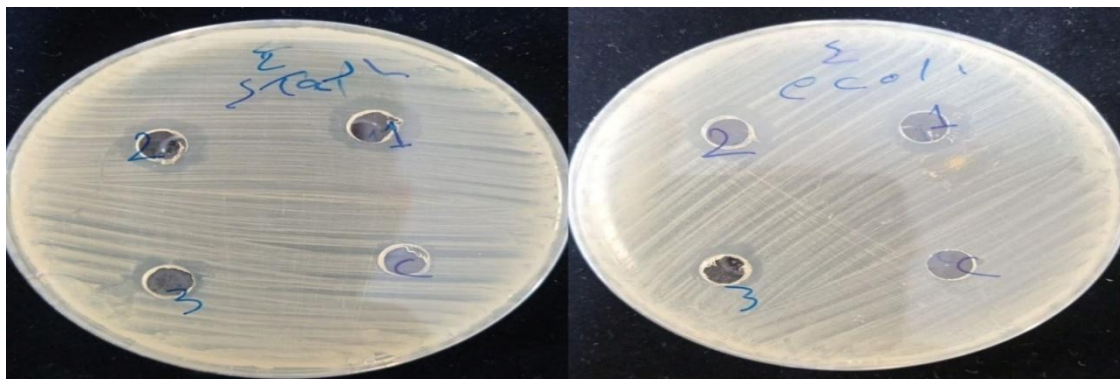


Figure 8. Antibacterial activity of the plant extract from sample A at three concentrations (100, 200, and 300 mg/mL) against *E. coli* and *S. aureus*, with 1% DMSO used as a negative control.

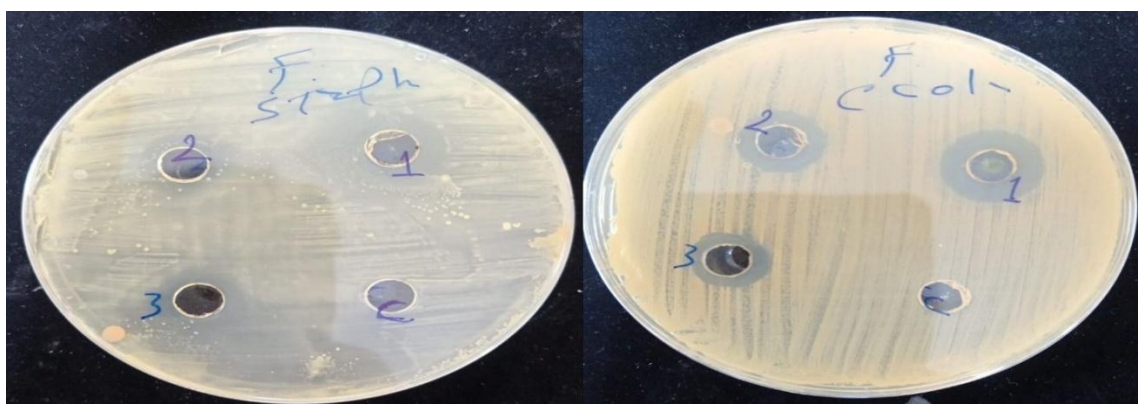


Figure 9. Antibacterial activity of the plant extract from sample A at three concentrations (100, 200, and 300 mg/mL) against *E. coli* and *S. aureus*, with 1% DMSO used as a negative control.

4. Discussion

The results of this study indicate that the use of jasmonic acid (JA) and plant growth regulators (2iP, 2,4-D, and NAA) effectively contributed to the regulation of secondary metabolic pathways within the callus cells of *Phoenix dactylifera* L. cv. Barhi. The interaction between these factors showed either stimulatory or inhibitory effects depending on the concentration applied. The data revealed that treatment B, which included a moderate concentration of JA, led to a noticeable increase in the number of detected chemical compounds. This is attributed to the role of JA in activating the gene expression of enzymes involved in the biosynthesis of phenolic and terpenoid compounds [20] as well as its ability to enhance secondary metabolism without negatively affecting cellular growth [29].

Growth regulators also exhibited a positive effect in enhancing the accumulation of secondary metabolites by stimulating cell division and promoting protein synthesis, which aligns with the findings of Rahman Al-Saedi *et al.* [30], Chavan *et al.* [31], and Abu Zaid [32]. The hormonal balance of growth regulators is considered a critical factor, as the highest concentrations of phenolics and flavonoids were recorded when moderate concentrations of 2,4-D, 2iP, and NAA were applied [12,33].

Conversely, the use of high concentrations of growth regulators may diminish the stimulatory effect of jasmonic acid, suggesting a hormonal interaction that alters the chemical composition of the compounds produced [34, 35].

The present study showed that alcoholic extracts obtained from the callus culture of *Phoenix dactylifera* L. cv. Barhi under the influence of growth regulators and jasmonic acid exhibited variable ability to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus*, reflecting the efficiency of these treatments in limiting the studied bacterial strains. This inhibitory activity is attributed to the presence of biologically active compounds, most notably phenolics and fatty acids, identified through GC-MS analysis.

Phenolic compounds are known for their ability to induce morphological changes in bacterial cells, such as damage to the cell wall and interference with biofilms formation, as well as inhibition of protein, ATP, and DNA synthesis via inhibition of the enzyme DNA gyrase. This is because of their antioxidant and antibacterial properties [36]. Free fatty acids (FFAs) exhibited a broad spectrum of biological activities through their direct effects on the cell membrane and disruption of vital processes such as the electron transport chain and oxidative phosphorylation, in addition to the production of toxic oxidative compounds [37].

Among the compounds identified by GC-MS and found in all samples, which may be responsible for the antibacterial activity 2,2'-Bioxirane stood out due to its antibacterial and antioxidant properties. Sharma *et al.* [38] documented its high proportion in the latex extract of *Euphorbia hirta*, along with clear bioactivity. α -Terpineol also demonstrated prominent activity against *E. coli* and *S. aureus* by affecting membrane fluidity and stimulating ROS production, leading to cell death [39]. Compound 2-Propanone, 1-hydroxy (acetol), showed inhibitory activity against bacteria, and its presence in the studied compounds suggests a potential contribution to the observed biological effect [40]. Benzophenone compounds exhibited broad activity against resistant microbial strains which is likely attributed to their chemical structure containing reactive groups such as peroxide bonds or chlorine atoms making them potential candidates for natural antimicrobial agents [41]. Dichloroxylenol exhibited strong disinfectant activity against *S. aureus* and *E. coli*, with rapid cell-killing efficiency, and its effect may vary depending on the structure of the bacterial cell wall [43, 44]. Butyrolactone showed notable activity against gram-positive bacteria such as *Bacillus subtilis*, due to its ability to interfere with vital processes within the bacterial cell [42]. Additionally, n-Hexadecanoic acid, a saturated fatty acid identified in all treatments, has been reported to possess antibacterial activity, particularly against *S. aureus*. Its mechanism of action involves disrupting the bacterial cell membrane and interfering with energy metabolism, which collectively impair cell viability [45-47].

Conclusion

The results of this study demonstrated that the interaction between plant growth regulators (2iP at 2 mg/l, 2,4-D at 8 mg/l, and 40 mg/l NAA) and jasmonic 2 mg/l significantly enhanced the production of bioactive compounds in the callus of *Phoenix dactylifera* L. cv. Barhi. This treatment recorded the highest number of chemical compounds identified by GC-MS, totaling 50 compounds, along with the highest percentage of total compounds among all the treatments (30.80%). Moreover, treatment B exhibited the strongest biological activity against *Staphylococcus aureus* and *Escherichia coli*, with inhibition zones exceeding those produced by the antibiotic disc Amoxyclav, which was used as a positive control. In contrast, treatment A showed the lowest number of compounds (32) and weakest antibacterial activity.

These findings confirm that the formulation applied in treatment B was the most effective for stimulating the biosynthesis of biologically active secondary metabolites, making it a promising system for enhancing the quality and bioactivity of plant extracts in tissue culture applications.

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Effect of Contact Time on the Efficiency of Nitrate Removal from Groundwater Using Recycled Tire Rubber as a Low-Cost Adsorbent

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Abstract. This study investigates the potential of recycled tire rubber granules as a sustainable and low-cost adsorbent for nitrate removal from contaminated groundwater. Laboratory-scale column systems were designed using rubber granules (1–3 mm, oven-dried at 60 °C for 24 h) and compared with a sand-only control. Synthetic groundwater solutions of sodium nitrate (NaNO_3) and potassium nitrate (KNO_3) were prepared at concentrations of 50–200 mg/L, and effluent samples were analyzed spectrophotometrically in accordance with APHA standard methods. The results showed that nitrate removal efficiency strongly depended on contact time, with maximum performance observed after four days. At 150 mg/L, removal efficiencies reached 79.1% for NaNO_3 and 79.7% for KNO_3 , while a similar efficiency (~80%) was achieved at 200 mg/L. Prolonged contact beyond four days resulted in reduced performance, likely due to adsorption site saturation or desorption. Kinetic analysis indicated diffusion-controlled adsorption, and the column design successfully simulated subsurface flow conditions. These findings demonstrate that recycled rubber can serve as an eco-friendly and effective alternative to conventional media, providing dual environmental benefits of groundwater remediation and waste tire recycling, thus contributing to circular economy strategies.

Keywords. Recycled Materials, Groundwater Remediation, Environmental Sustainability, Eco-friendly Adsorbent, Contact time.

1. Introduction

Groundwater contamination, particularly nitrate removal, is a major hazard to the environment and human health. This study explores innovative and sustainable techniques for addressing nitrate pollution in groundwater, especially using recycled rubber from discarded tires. This study assessed the usefulness of several methods for using recycled rubber as a nitrate adsorbent based on experimental and field performance [1]. Nitrate ions play a crucial role in the nitrogen cycle and are abundant in nature. This highly soluble water contaminant poses a severe threat to global drinking water supplies and aquatic environments because it causes eutrophication [2]. Nitrate levels in surface water and groundwater are rising worldwide, endangering both human health and aquatic ecosystems. The nitrate concentrations were higher in areas with considerable agricultural activities. Soil and geological conditions affect the natural nitrate content of the

groundwater [3]. Nitrate pollution in drinking water ($\text{NO}_3^- > 10 \text{ N-mg/L}$) is mostly caused by agricultural and industrial operations and has serious health consequences, including methemoglobinemia, cancer, and thyroid issues [4]. Nitrogen in the form of nitrate in water can be toxic to humans and other living organisms. Abortions, delivery problems, an increased risk of hemolytic anemia and gastric cancer, gastrointestinal system degradation, ulcers, and teratogenic consequences have all been related to excessive nitrate levels in drinking water. Therefore, it is necessary to maintain track of the nitrate levels in groundwater. The World Health Organization (WHO) established drinking water thresholds of 15 mg/L for children (infants) and 50 mg/L for adults [5]. This is because nitrate has a deleterious effect on human health. There are several methods for eliminating nitrates from water, including biological, chemical, and physical processes. Physicochemical treatments, such as reverse osmosis, ion exchange, adsorption, electro-dialysis, and catalytic processes, may successfully remove nitrate ions. However, their utilization is constrained owing to elevated operational expenses. Although slow, biological denitrification is an efficient wastewater treatment method that requires disposal of activated sludge. Adsorption is the dominant method for industrial-scale applications [6]. Nitrate levels in water bodies pose serious health risks, especially in vulnerable groups such as children, pregnant women, and babies. Potential health risks associated with nitrate exposure include myocardial infarction, cardiovascular hypertrophy, heart attacks, heart disease, methemoglobinemia (blue baby syndrome), and retinal lipid peroxidation. The International Agency for Research on Cancer (IARC) has identified nitrate as a likely human carcinogen [7]. The ability of fish and other organisms to survive, grow, and reproduce may be affected by their presence in waterbodies. Soil can be degraded using polluted wastewater. Pollution of groundwater sources. This may damage agricultural and drinking water supplies, with long-term consequences for the environment [8]. Adsorption is a cost-effective and efficient method for the removal of nitrates from water. It is also straightforward to implement and execute. Several adsorbents have been studied for nitrate removal [9]. Current research focuses on developing environmentally sound nitrate removal technologies and expanding our understanding of the processes that lead to nitrate production and contamination of groundwater [10]. Tire disposal and recycling at the end of its useful life represent serious environmental issues for both the industry and academia. This result can be attributed to several crucial variables. Tires exhibit excellent performance. Tires are manufactured with high-quality components, including rubber compounds, steel wires, and textile cables, which makes recycling discarded tires difficult. The most challenging aspect of recycling scrap tires is vulcanization, which involves a cross-linked rubber structure [11]. Rubber is classified according to its usage and saturation levels. Common variations include synthetic rubbers, natural rubber, nitrile-butadiene rubber, ethylene-propylene-diene monomers, silicone rubber, and polyurethane. Over the past five years, the global rubber output has reached 25 million tons per year. Polymeric rubber accounts for approximately 50% of the tire material [12]. The contact time is critical for determining the adsorbent efficiency. It refers to the average contact time needed to reach pollutant equilibrium between the adsorbent surface and the aqueous solution. Additionally, it allows for kinetic analysis of pollutant adsorption, which leads to a better understanding of its mechanics [13].

The current study is unique in that it uses a laboratory-scale vertical column system to replicate subsurface flow. Furthermore, a direct comparison with a sand-only control column was used to assess the additional value of rubber medium. Furthermore, this work gives kinetic insights into contact time and diffusion-controlled adsorption, which are rarely seen in column-based assessments. These qualities distinguish the current study and make it suitable for groundwater treatment.

The goal of this study was to evaluate the impact of contact time on the efficiency of nitrate removal from contaminated ground water through adsorption onto crumb rubber, a low-cost, environmentally friendly adsorbent derived from scrap tires. This study was performed under laboratory conditions with a column configuration to simulate the subsurface flow. Therefore, the aim of this work was to examine the adsorption performance at various contact times to optimize the operational conditions for potential use in the sustainable remediation of groundwater.

2. Materials and System Design

2.1. Materials

The main adsorbent material used in this study was recycled rubber granules, generated from waste vehicle tyres. Given times for the washed rubber was transferred to a sieve and to the oven at 60°C for 24 h, and sieved to produce a uniform particle size of (1-3) mm. To mimic impaired groundwater, we prepared synthetic nitrate-polluted groundwater by dissolving analytical-grade KNO_3 in deionized water. The solutions were prepared with distilled water, and all reagents and solvents were of analytical grade. Nitrate analysis was conducted using a Spectrophotometer (UV-VIS Double Beam PC) UVD-3000 Labomed, Inc., USA, in line with APHA Standard Method 4500- NO_3^- B. To imitate subsurface infiltration, the influent flow rate across both columns was set to 20 mL/min with a peristaltic pump. Before column studies, all influent nitrate solutions were adjusted to a pH of 7.0 ± 0.2 using 0.1 M HCl or NaOH.

2.2. Experimental System Design

A laboratory-scale column system was developed to evaluate the influence of contact time on nitrate removal from polluted water solutions using recycled rubber as the candidate filtering medium. The experimental setup was composed of two vertically standing (approximately 100 cm high) strong PVC pipes with internal diameter of 5 cm. The two columns were connected to a reservoir containing synthetic nitrate-contaminated water. The first column, often known as the Rubber Column, was filled from the bottom with the following layers:

- 10cm gritty gravel.
- A layer of 5 cm coarse-sand and 10 cm fine-sand.
- 30 cm of recycled raw rubber granules (adsorbent).
- Add a 10cm layer of fine gravel on top.

The Control Column was filled in the same manner as the Rubber Column, but with 30 cm of fine sand instead of rubber. This control configuration was used to analyze the contribution of recycled rubber to nitrate removal by comparing it with a traditional sand-based filtering medium, as shown in Figure 1. Water from the storage tank flowed down both columns under gravity and passed through the filter layers sequentially. Effluent samples were collected at different time points to assess the impact of contact duration on the efficiency of nitrate removal. To guarantee data reliability and repeatability, the system was run in ambient laboratory settings and all tests were performed in triplicate.

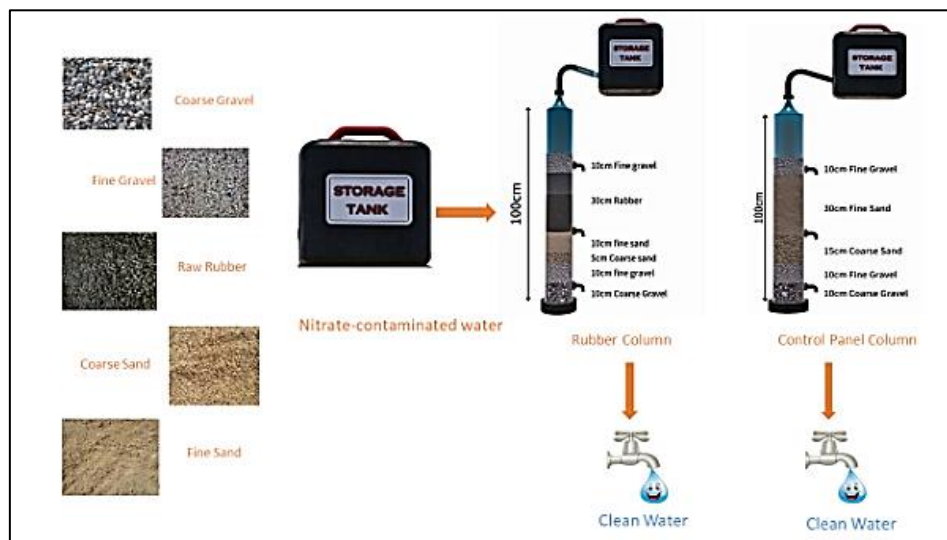


Figure 1. Schematic cross-sectional view of the laboratory-scale column system showing layers' configuration.

2.3. Analytical Methods

A UV-Vis Spectrophotometer (Model: [Insert Model Number], Manufacturer: [Insert Manufacturer]) was employed to measure the concentration of nitrate (NO_3^-) in water samples from the rubber and control columns. The analysis followed the APHA's Standard Methods for the Examination of Water and Wastewater (Method 4500- NO_3^- B). The water samples were filtered to eliminate suspended particles prior to examination. To ensure accuracy, a calibration curve was constructed using nitrate standard solutions at established concentrations. The absorbance was measured at 220 nm, which corresponds to the maximum absorption of nitrate ions. To avoid interference from organic materials, which may also absorb at 220 nm, a secondary measurement was performed at 275 nm, and a correction was performed in accordance with APHA recommendations [14]. All measurements were taken in triplicate, and the average results were used to assess the nitrate removal effectiveness at different contact periods. The removal percentage was estimated using the following equation:

$$\text{Nitrate Removal (\%)} = ((C_0 - C_t) / C_0) \times 100\%$$

Where: C_0 the initial concentration of nitrate (mg/L), and C_t the concentration at time t after passing through the column.

The column experiments were conducted at a flow rate of 1.5–2 mL/min, corresponding to a hydraulic retention time (HRT) of 1–8 days, which is critical for evaluating the effect of contact time on nitrate removal efficiency. This flow rate range was selected to simulate extended contact times relevant to subsurface groundwater conditions while maintaining a manageable laboratory-scale setup.

3. Results and Discussion

Using a neutral pH of 7 and a 100 mL solution volume, batch tests were conducted to evaluate the impact of contact length on nitrate removal performance. A 25°C controlled ambient temperature, a 150 rpm shaking speed, 5% of the weight of the solution volume as a dose of rubber sorbent, and initial nitrate concentrations of 50, 100, 150, and 200 mg/L for both sodium and potassium nitrate were all part of the test setup. The impact on the nitrate adsorption performance was

studied by methodically adjusting the contact period. Figure 2 shows that the nitrate removal efficacy of NaNO_3 (sodium nitrate) and KNO_3 (potassium nitrate) solutions is enhanced with longer contact time. At 6 days, KNO_3 had a higher clearance rate of 38% than NaNO_3 , which was just 27%. The observed difference may be the result of nitrate ion exchange with the recycled rubber surface being facilitated by the higher ionic radius and mobility of K^+ . Previous research [15], [16] has indicated that extended contact durations enhance adsorption by enabling more nitrate ions to diffuse and bind to active sites, which is in line with the prevailing trend. Recycled rubber is a sustainable adsorbent that has shown promise for inexpensive groundwater remediation.

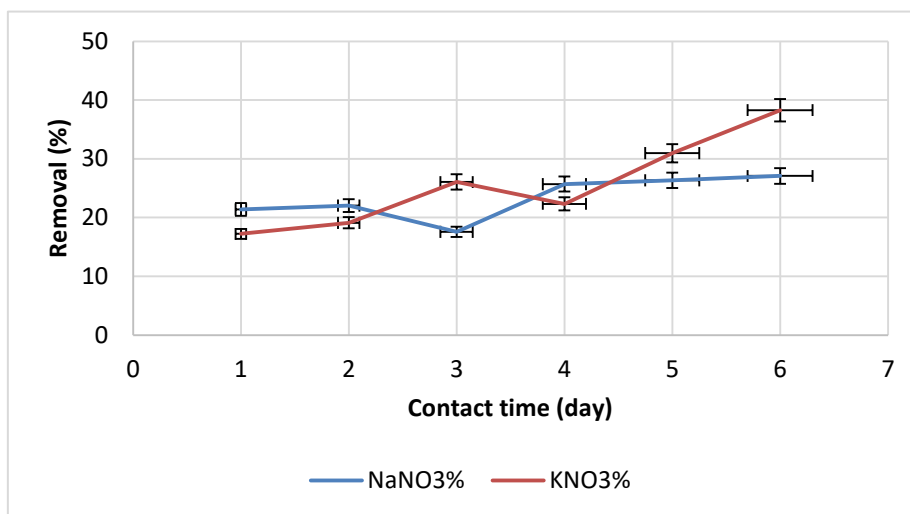


Figure 2. The influence of nitrate removal contact time at 50 mg/L concentration.

When using recycled rubber as an adsorbent, the results showed that the efficacy of nitrate removal was significantly affected by contact duration. As the contact duration increased up to 4 hours, the removal efficiency of NaNO_3 solutions at a concentration of 3% and KNO_3 solutions at a concentration of 3% reached 79.1% and 79.7%, respectively. The longer the time for which nitrate ions interact with the accessible adsorption sites on the rubber surface, the better the result. It is probable that active site saturation or desorption caused a significant decrease in the efficacy of both salts in removing contaminants after 5 days. According to these results, as shown in Figure 3, the optimal contact length for nitrate removal is approximately four days, which strikes a good balance between performance and operating economy.

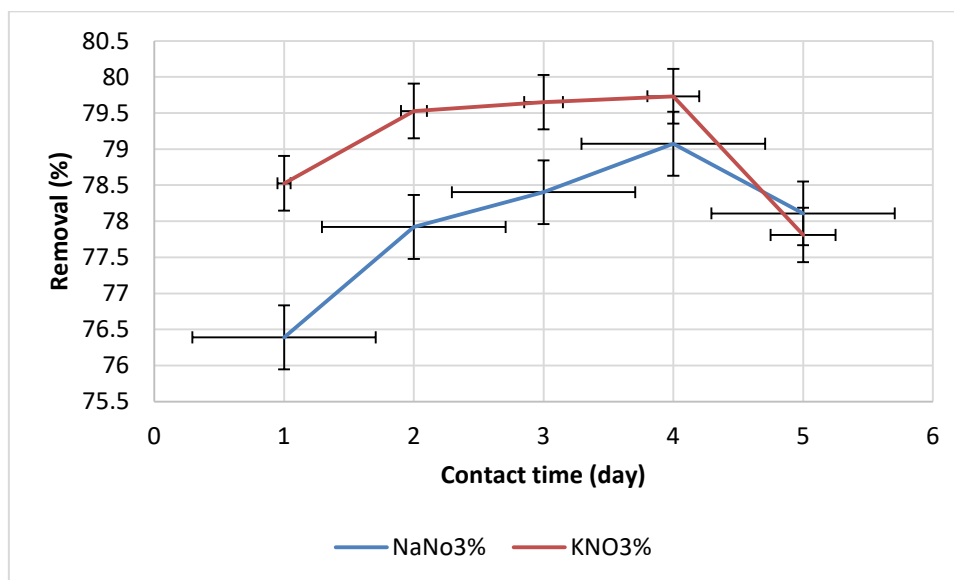


Figure 3. The influence of nitrate removal contact time at 100 mg/L concentration.

Figure 4 shows that the clearance efficiencies of both the NaNO₃ and KNO₃ solutions at 3% concentrations increased with contact time up to 4 days, reaching a maximum of 72.8% and 73.19%, respectively, at a concentration of 150 mg/L.

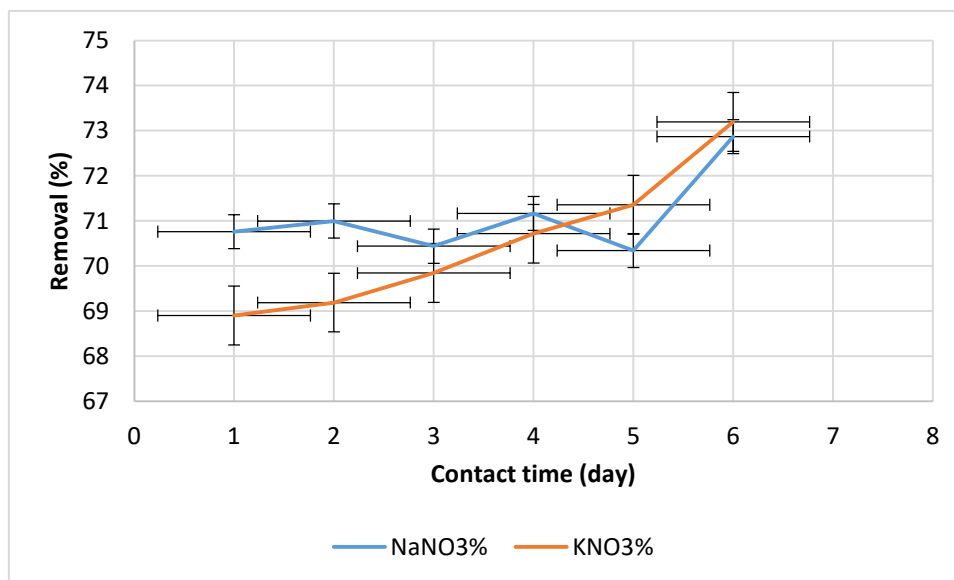


Figure 4. The influence of nitrate removal contact time at 150 mg/L concentration.

Fig.5 shows that the removal efficiencies of NaNO₃ (at 200 ppm) and KNO₃ (at 3% concentration) solutions increased with contact time up to 4 d, reaching 79.76% and 79.8%, respectively. This improvement occurs because the nitrate ions have more time to interact with the available adsorption sites on the rubber surface. After five days, the removal efficiency of the salts dropped significantly, either as a result of desorption effects or active site saturation. These results suggest that an approximately 4-day contact period ensures the maximum removal of nitrate and maintains performance and operating convenience. These results are in line with those

reported in [17], [18]. These trends show the correlation between contact time and nitrate removal, and are viable at different concentrations for both nitrate salts. Overall, the findings are encouraging and suggest the applicability of raw recycled rubber as a cost-effective and environmentally friendly nitrate sorbent, particularly for groundwater treatment.

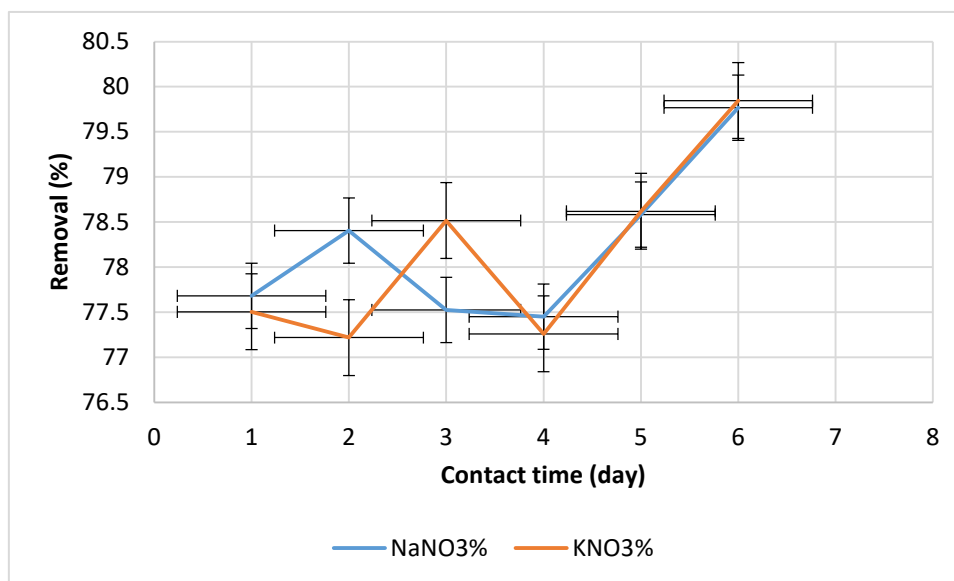


Figure 5. The influence of nitrate removal contact time at 200 mg/L concentration.

Conclusions

This study demonstrated that recycled rubber obtained from waste tires can be effectively and inexpensively used as a long-term nitrate adsorbent for extracting nitrates from contaminated groundwater. It is concluded that the removal efficiency is closely related to the contact time; the removal efficiency increases slowly over time and reaches its highest value on day 4, eventually decreasing because the adsorption site is saturated or desorbed. The removal efficiencies by NaNO_3 and KNO_3 solutions were the same, which reached a maximum of approximately 80% for lab-controlled conditions in the study. Ion transport and surface contact in the adsorption process were observed, suggesting the importance of the kinetic and equilibrium parameters in the design. Contact time was demonstrated to be essential for enhancing the efficiency of nitrates removal from groundwater using a recycled rubber medium in the current investigation. The maximum removal of NaNO_3 and KNO_3 solutions was 79.1% and 79.7%, respectively, at 150 mg/L after 4 days of contact. In the past, there was something of a downturn, which can probably be attributed to media saturation. These findings indicate the utilization of recycled tire rubber as a sustainable and cost-effective adsorbent for nitrate removal, allowing for environmentally acceptable groundwater treatment options. This main trend is consistent with previous investigations [19]. From an engineering perspective, the column configuration successfully simulated subsurface flow and offered valuable insights into the performance of recycled rubber as a filter medium, relative to standard sand technology. All graphical findings are shown as mean values from three studies. Error bars represent standard deviations, and the small overlap between error ranges indicates statistical significance, as validated by ANOVA and LSD post-hoc tests at $p < 0.05$. The experimental data were statistically validated using one-way ANOVA and LSD post hoc testing ($p < 0.05$). The results showed that pH, contact length, starting nitrate concentration, and rubber

dose all had a statistically significant effect on nitrate removal efficiency. The best circumstances were discovered as pH 6-8, contact length of roughly 96 hours, rubber dose of 5% w/v, and starting nitrate content of 100-150 mg/L. The modest standard deviations across triplicates validated the system's dependability and repeatability. These findings emphasize the necessity of process optimization and show that the observed removal efficiencies are constant and statistically significant, validating the use of recycled rubber as a sustainable and efficient adsorbent for groundwater remediation. This finding demonstrates the potential of directly incorporating recycled material (rubber) into field-applicable groundwater remediation units, providing an environmentally preferable alternative solution to the critical problem at the end of life of tires.

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The Influence of Mineral and Biological Fertilizers on the Level of Total Organic Carbon, Nitrogen, and the C/N Ratio in the (*Zea mays L.*) Rhizosphere and Bulk Soils, and on Soil Sustainability

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Abstract. This study aimed to investigate the effects of biofertilizers and mineral fertilizers on the levels of total nitrogen, organic carbon, and nitrogen-to-carbon ratio in soil. A field experiment was performed on a silty- clay soil. The study treatments included a control treatment, urea at 250 kg N ha⁻¹, ammonium sulfate at 200 kg h⁻¹, BioHealth at 4-5 kg h⁻¹, liquid EM at 400 L h⁻¹, and a fertilizer combination consisting of one-quarter of the recommended dosage of each fertilizer. Total nitrogen and organic carbon were assessed in the rhizosphere and rhizosphere soils at 70 and 100 days post-planting to evaluate the impact of minerals and biofertilizers on their soil availability. The findings indicated that the combined treatment produced the maximum total nitrogen concentration in rhizosphere soil at 70- and 100-days post-planting. In the rhizosphere soil, urea treatment exceeded the total nitrogen concentration at 70- and 100-days post-planting. The findings indicated a reduction in total nitrogen concentration in both the rhizosphere and rhizosphere soils after 70 and 100 d . The findings indicated that the combination of biofertilizers and mineral fertilizers surpassed the organic carbon concentration in both the rhizosphere and rhizosphere soils 70 d post-planting. At 100 days, urea treatment surpassed both the rhizosphere and rhizosphere soils. The organic carbon concentration in the rhizosphere was markedly lower than that in the rhizosphere soils across all treatments, except for urea, as the growth duration increased. Organic carbon levels increased in all rhizosphere treatments, except for the combined fertilizer treatment. The results indicated that N: C ratios decreased across all treatments relative to the control, with values declining as the growth period extended (100 d post-planting), except for the urea treatment. The ratios fluctuated based on the type of carbon and nitrogen source ued.

Keywords. Total nitrogen, organic carbon, c/n, Rhizosphere, Bio fertilizer, Mineral fertilizer, Soil sustainability.

1. Introduction

The zone of soil influenced by the biological activity of plant roots, known as the rhizosphere, is crucial for plant development and enhancing soil fertility. This region has unique physical, chemical, and biological characteristics that are in contrast to those of the undisturbed soil. These

qualities encompass a reduced response rate attributable to elevated CO₂ generation from microbial and plant root respiration. Research has indicated that this area is a crucial location for biological interactions between microorganisms and the soil environment (1). When used judiciously, mineral fertilizers significantly enhance agricultural productivity. Inadequate treatment may result in delayed maturation and impact crop quality (2). Biofertilizers can help reduce the reliance on chemical fertilizers. They serve as a method for land development with minimal environmental contamination while also supplying plants with nutrients and growth-enhancing chemicals (3). Biofertilizers are an effective and eco-friendly alternative, comprising microbial bioproducts that contain millions of microorganisms suitable for application to seeds, soil, or both. These microbes can promote plant development by increasing the availability of elements in the rhizosphere, especially nitrogen and phosphorus, from non-renewable sources (4). Maize is a soil-stressing crop because of its substantial nutrient requirements, which are derived from the soil for vegetative development and grain production (5,6). Crop productivity is influenced by inadequate soil fertility, necessitating the application of mineral fertilizers to fulfil the nutritional needs of

crops (7). Nitrogen is a vital nutrient and a significant factor in determining the plant production per unit area. Therefore, it is crucial to fortify and develop root systems. Nitrogen shortage diminishes leaf area (8). (9) demonstrated that climatic conditions and the carbon-to-nitrogen (C:N) ratio in plant residues directly impact the soil's capacity to sequester organic carbon. Soil sustainability is essential for ensuring sustainable agricultural output and conserving ecosystems, as it encompasses maintaining fertility and reducing environmental degradation. This sustainability depends on coordinated resource management and preservation of organic matter (11). This study aimed to investigate the impact of mineral and biological fertilizers on the concentrations of total nitrogen, organic carbon, and nitrogen-to-carbon ratio within and outside the maize rhizosphere.

2. Materials and Methods

The research was conducted in a field located in the Al-Nuriyah sub district of Al-Shafi'iyah District, Diwaniyah Governorate, during the autumn of 2024. Yellow corn was cultivated in a silty clay soil. The study soil was partitioned into three sectors, each including six 3 × 3 m plots (treatments) serving as experimental units. A separation of 1 m was maintained between the sectors and 1 m between

the experimental units to inhibit the movement of water and fertilizers between the treatments. The experiment was structured as a randomized complete block design (RCBD) with three replicates. Each sector consisted of six treatments that were, randomly allocated across 18 units. The experiment consisted of the following treatments:

- Control treatment
- Urea fertilizer (46% N) was applied 10 d after planting at a level of 250.
- Ammonium sulfate fertilizer 21% N, 24% S, 10 days after planting at a rate of 200 kg ha⁻¹.
- Bio Health fertilizer was applied 10 days after planting at a level of 4-5 kg ha⁻¹. Humintech Germany prepared the health fertilizer levels.
- An effective microorganism liquid biofertilizer was manually sprayed. One liter of this fertilizer was diluted in 4 liters of water. This process was carried out using a 16-liter hand sprayer, with five sprays each spraying (400 liters ha⁻¹). It is a mixture of certain fungi, bacteria, humic acid, and seaweed in the form of water-soluble granules, and contains the components listed in Table 1.

- Fertilizer mixture (a quarter of the recommended amount of each fertilizer).

Table 1. Components of Biohealth Fertilizer as per the Manufacturer (Humintech, Germany).

	Contents	Quantity
Fungi	Trichoderma	5%
Bacteria	<i>Bacillus subtilis</i>	5%
Organic matter	Humic acid	75%
Organic matter	Seaweed	5%
Organic matter	Fulvic acid	65%
Elements	Water-soluble potassium K ₂ O	11%
Elements	Boron	15 mg kg ⁻¹

Yellow corn (*Zea mays* L.) seeds were sown in rows on August 17, 2024. The inter-row spacing was 75 cm, and the inter-hole distance was 25 cm, with four rows allocated to each plot. Three seeds were sown in each hole and thinned to one following germination. Each row contained 12 plants, With a total of 48 plants in the experimental unit. The corn stem borer was managed with a granular diazinon herbicide by, applying one grain in the middle of each leaf. Manual weeding was performed five times during the growing season. The yellow corn crop was harvested after the appearance of maturity signs, resulting from ear desiccation, on December 19, 2024.

- Total nitrogen: Total nitrogen was quantified using the Kjeldahl technique, as described previously(11).
- Organic Carbon: Soil organic carbon was calculated from organic matter measurements using a conversion factor of 1.72 (12).
- C/N ratio: The C/N Ratio was calculated by dividing organic carbon by total nitrogen. Operation with laboratories at Mosul University, Ethical Approval No. um.VET.2021.5.,

2.1. Statistical Analysis

SAS Statistical Analysis System (SAS) software was used to examine the data. The data were analyzed using a randomized complete block design (RCBD). Substantial differences between means were assessed using the least significant difference (LSD) test at a significance threshold of 0.05.

3. Results and Discussion

3.1. Total Nitrogen Level in the Study Soil 70 Days Post-Planting

Table 2 presents the effect of incorporating mineral fertilizer, biofertilizer, and a combination of both on the total nitrogen content in the rhizosphere and bulk soils 70 days post-planting. The statistical analysis results indicated significant differences a significance level of 0.05. All interventions surpassed control therapy. The maximum total nitrogen content in the bulk soil was attained with the ammonium sulfate treatment (5053 mg kg⁻¹). Ammonium sulfate fertilizer increased the total nitrogen content compared to other fertilizers such as urea and ammonium nitrate. This may be attributed to its capacity to reduced nitrogen loss (13). The use of biofertilizers in conjunction with mineral fertilizers in the rhizosphere soil resulted in the highest nitrogen concentration of 3426.88 mg kg⁻¹. Research indicates that combining biofertilizers with mineral fertilizers results in a nitrogen concentration increase of up to 63.68% compared with urea (14).

Table 2. Impact of fertilizer type on total nitrogen content (mg kg^{-1}) in both rhizosphere and non-rhizosphere soil 70 days post-planting.

Treatments	Soil Zone	
	Rhizosphere soil	Bulk soil
Control Treatment	344.90	368.14
Urea	2767.88	3172.34
Ammonium Sulfate	2223.98	5053.86
Biohealth	1593.48	3873.49
Effective Microorganisms	1281.28	2936.85
Bio-Mineral Fertilizer Combination	3426.88	4769.22
LSD 0.05	80.686	110.874

Mineral fertilizers (urea and ammonium sulfate) surpassed biofertilizers (BioHealth and EM) in total nitrogen content in both rhizosphere and rhizosphere soils. This aligns with the findings of (15), who showed that mineral fertilizers result in elevated levels of ammonium and nitrate ions relative to organic fertilizers. The urea treatment produced a total nitrogen content of $2767.88 \text{ mg kg}^{-1}$ in the rhizosphere soil, surpassing that of the ammonium sulfate treatment. Research has demonstrated that urea application elevates soil nitrogen levels by 20–45% relative to the control treatment (16). The findings indicated that total nitrogen concentration was higher in rhizosphere soils than in rhizosphere soils across all treatments. This may be because the rhizosphere is a biologically active environment with a high density of microorganisms and roots that swiftly absorb nitrogen, there by depleting a significant percentage of nitrogen in this region (17).

3.2. Total Nitrogen Level in the Study Soil 100 Days Post-Planting

Table 3 presents the impact of incorporating biofertilizer, mineral fertilizer, and a combination of both on the total nitrogen content in the rhizosphere and surrounding soil 100 d after planting. The statistical analysis results indicated significant differences a 0.05 significance level of 0.05. All treatments surpassed the control treatment, with the highest total nitrogen content in the rhizosphere soil recorded in the combination of biofertilizers and mineral fertilizers, measuring $3390.32 \text{ mg kg}^{-1}$. The maximum nitrogen content in the rhizosphere soil was attained with ammonium sulfate, measuring $5077.70 \text{ mg kg}^{-1}$. This is because ammonium sulfate fertilizer is a direct source of total nitrogen (18). This aligns with the findings of (19), who demonstrated that the use of mineral fertilizer, whether alone or in combination with biofertilizer or organic fertilizer, resulted in a substantial increase in ammonium content. The biofertilizer (EM) treatment resulted in the lowest total nitrogen content in the rhizosphere soil compared to other fertilizers. The measured nitrogen content was $1246.77 \text{ mg kg}^{-1}$. This may result from microbes fixing approximately 60% of the nitrogen that enters the soil (19,20,21). The data from Tables 2 and 3, together with Figure 1, indicate that the total nitrogen content in the rhizosphere soil diminished after 70 and 100 days across all treatments. This may result from absorption by plants and the loss of nitrogen from the soil via volatilization (22), nitrification (23), fixation in organic matter or clay minerals, or utilization by soil organisms when carbon is available as an energy and growth source. This aligns with the findings of (24).

Table 3. Impact of fertilizer type on total nitrogen content (mg kg^{-1}) in the rhizosphere and bulk soils of maize 100 days post-planting.

Treatments	Soil Zone	
	Rhizosphere soil	Bulk soil
Control Treatment	326.05	394.15
Urea	2731.47	3194.21
Ammonium Sulfate	2191.07	5077.70

Treatments	Soil Zone	
	Rhizosphere soil	Bulk soil
Biohealth	1553.73	3896.72
Effective Microorganisms	1246.77	2962.25
Bio-Mineral Fertilizer Combination	3390.32	4796.47
LSD 0.05	81.795	114.181

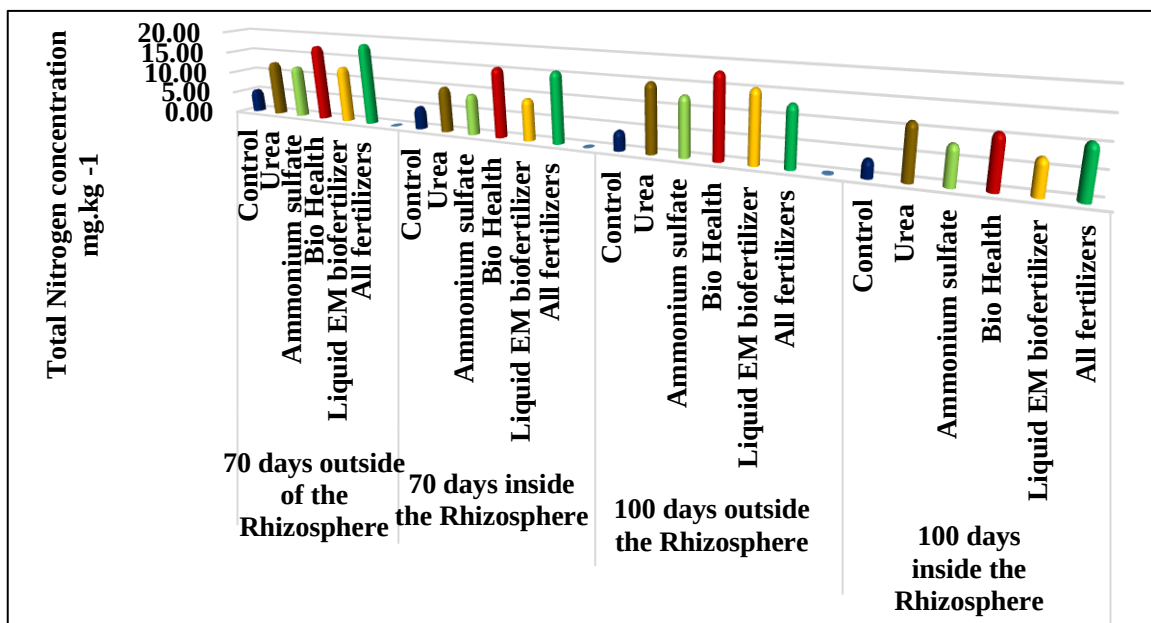


Figure 1. Impact of fertilizer type on nitrogen content in rhizosphere and rhizosphere soils at 70- and 100-days post-planting (mg kg^{-1}).

3.3. Organic Carbon Level in the Study Soil 70 Days Post-Planting

Table 4 presents the impact of mineral fertilizers, biofertilizers, and their combined application on the organic carbon content in both rhizosphere and rhizosphere soils 70 d post-planting. The statistical analysis results indicated significant differences a 0.05 significance level of 0.05. All treatments surpassed the control treatment in both rhizosphere and rhizosphere soils, with carbon contents of 5.14 g kg^{-1} and 5.33 g kg^{-1} , respectively. The maximum organic carbon concentration in the rhizosphere and rhizosphere soils was attained with the combination of all biofertilizers and mineral fertilizers, reaching 15.82 and 18.71 g kg^{-1} , respectively. Research has demonstrated that this combination attains equilibrium between nutrients and biological activity (25,26).

Table 4. Impact of fertilizer type on organic carbon content (g kg^{-1}) in the rhizosphere and bulk soils 70 days post-planting.

Treatments	Soil Zone	
	Rhizosphere soil	Bulk soil
Control Treatment	5.14	5.33
Urea	10.21	12.67
Ammonium Sulfate	9.13	12.02
Biohealth	15.66	17.46
Effective Microorganisms	9.35	12.88
Bio-Mineral Fertilizer Combination	15.82	18.71
LSD 0.05	3.249	3.434

The results also showed an increase in organic carbon concentrations with the urea treatment, reaching 10.21 and 12.02 g kg⁻¹ for the rhizosphere and rhizosphere soils, respectively. This was attributed to the fact that urea is a rich source of nitrogen. This stimulates root growth and consequently increases root exudates, such as amino acids and sugars, which are considered carbon-rich (27). The BioHealth treatment produced a higher total carbon concentration than the EM biofertilizer treatment. This is because the fertilizer contains organic matter, specifically humic acid (75%), seaweed (5%), and other carbon-rich materials (20%).

3.4. Organic Carbon Level in the Study Soil 100 Days Post-Planting

Table 5 presents the impact of mineral fertilizers, biofertilizers, and their combined application on the organic carbon concentration in both rhizosphere and Bulk soils, measured 100 d post-planting. The statistical analysis results indicated significant differences a significance level of 0.05. All treatments surpassed the control treatment in both rhizosphere and rhizosphere soils, with carbon contents of 3.79 and 4.35 g kg⁻¹ in the rhizosphere and rhizosphere soils, respectively. The urea treatment resulted in the highest organic carbon content in both the rhizosphere and rhizosphere soils, measuring 11.65 and 15.10 g kg⁻¹, respectively. This aligns with the findings of (28) in their research on maize plants at the maturity stage. The ammonium sulfate treatment resulted in reduced carbon content in both the rhizosphere and rhizosphere soils, measuring 8.22 and 12.94 g kg⁻¹, respectively, compared to other fertilizer types. Ammonium sulfate may enhance the breakdown of organic materials in the soil, leading to increased microbial activity, which consumes carbon as an energy source.

Table 5. Impact of fertilizer type on organic carbon content (g kg⁻¹) in the rhizosphere and bulk soils 100 days post-planting.

Treatments	Soil Zone	
	Rhizosphere soil	Bulk soil
Control Treatment	3.79	4.35
Urea	11.65	15.10
Ammonium Sulfate	8.22	12.94
Biohealth	11.02	18.21
Effective Microorganisms	7.29	15.48
Bio-Mineral Fertilizer Combination	10.75	13.08
LSD0.05	1.841	7.610

The data presented in Tables 4 and 5, as well as Figure 2, indicate that the organic carbon concentration in the rhizosphere was consistently lower than that in the rhizosphere soil across all treatments, except for urea, as the growth period increased. Simultaneously, organic carbon levels increased in all treatments outside the rhizosphere. Research has shown that the rhizosphere is a biologically dynamic ecosystem. Microorganisms use carbon as an energy source, resulting in a reduction in their concentrations in this area (29).

These findings validate that biological treatments, particularly when combined with mineral fertilizers, represent optimal techniques for enhancing organic carbon levels in the soil, thereby promoting soil fertility and sustainability. Biofertilizers enhance organic carbon levels, illustrating their capacity to bioactivate and accumulate organic matter in the soil. Conversely, mineral fertilizers exhibited fluctuating levels, although they showed reduced stability over time. This indicates that incorporating biofertilizers into the agricultural framework is effective.

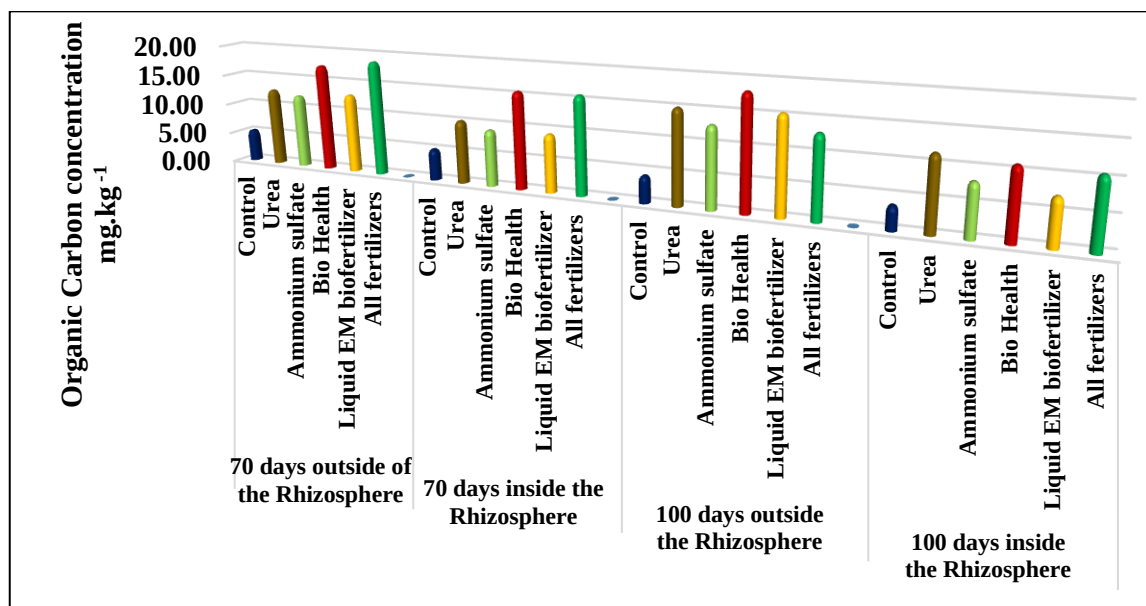


Figure 2. Impact of fertilizer type on carbon content in rhizosphere and rhizosphere soils at 70- and 100-days post-planting (mg kg^{-1}).

3.5. Carbon-to-Nitrogen Ratio in the Study Soil 70 Days Post-Planting

Table 6 presents the impact of mineral fertilizers, biofertilizers, and their combined application on the carbon-to-nitrogen ratio of both rhizosphere and rhizosphere soils 70 d post-planting. The statistical analysis results indicated significant differences a significance level of 0.05. The control treatment surpassed the other treatments in terms of the carbon-to-nitrogen ratio, with values of 15.09 and 14.50 for the rhizosphere and rhizosphere soils, respectively. This is due to the lack of a readily available nitrogen supply and the reduced activity of microbes. This results in the buildup of undecomposed organic carbon, which depends on the inherent organic matter content of the

soil. A reduction in the C/N ratio was also noted following urea and ammonium sulfate treatments. This was due to the presence of highly soluble and absorbable nitrogen (in the form of nitrate and ammonium) in these fertilizers, while organic carbon remained stable. Consequently, organisms employ nitrogen for in metabolic activities and breakdown of organic materials, whereas carbon is released as CO_2 (30).

Table 6. Impact of fertilizer type on the carbon-to-nitrogen ratio 70 days post-planting.

Treatments	Soil Zone	
	Rhizosphere soil	Bulk soil
Control Treatment	15.09	14.50
Urea	3.69	3.99
Ammonium Sulfate	4.10	2.38
Biohealth	9.84	4.52
Effective Microorganisms	7.30	4.38
Bio-Mineral Fertilizer Combination	4.64	3.93
LSD 0.05	2.919	1.124

The ratios obtained for the rhizosphere and rhizosphere soils were 4.64 and 3.92, respectively, when a combination of biofertilizers and mineral fertilizers was used. This ratio is inferior to that

of biofertilizers, and superior to that of mineral fertilizers. This equilibrium may result from the accessibility of nitrogen from mineral fertilizers and the provision of carbon from biofertilizers.

3.6. Carbon-to-Nitrogen Ratio in the Study Soil 100 Days Post-Planting

Table 7 presents the impact of mineral fertilizers, biofertilizers, and their combined application on the carbon-to-nitrogen ratio in both rhizosphere and rhizosphere soils, measured 100 d post-planting. The statistical analysis results indicated significant differences a significance level of 0.05. The control treatment surpassed the other treatments in the carbon-to-nitrogen ratio, with values of 11.74 and 11.17 for the rhizosphere and rhizosphere soils, respectively. The findings indicated a reduction in the C/N ratio when biofertilized with the BioHealth treatment (4.67 and 7.10) for both rhizosphere and rhizosphere soils. This signifies the existence of biological activity in the rhizosphere. This aligns with the findings of (27), who show that biofertilization accelerates carbon mineralization and promotes nitrogen availability, thereby decreasing the C/N ratio.

Table 7. Impact of fertilizer type on the carbon to nitrogen ratio 100 days post-planting.

Treatments	Soil Zone	
	Rhizosphere soil	Bulk soil
Control Treatment	11.17	11.74
Urea	4.72	4.27
Ammonium Sulfate	2.56	3.75
Biohealth	4.67	7.10
Effective Microorganisms	5.21	5.85
Bio-Mineral Fertilizer Combination	2.72	3.18
LSD 0.05	2.695	1.623

The reduction in the C/N ratio with mineral fertilizers (urea and ammonium sulfate) in both rhizosphere and non-rhizosphere soils aligns with the findings of (31) in their study on maize and sunflowers throughout the maturity stage. The findings in the rhizosphere demonstrate that the C/N ratio consistently falls below that of the Centre (NFDC) Review Report 1/ 2005, Islamabad. surrounding environment, signifying the accelerated breakdown of organic waste and enhanced efficacy of fertilizers, particularly biofertilizers. The carbon-to-nitrogen ratios were elevated in soils outside the rhizosphere particularly in the control treatment, indicating a reduced rate of organic matter breakdown.

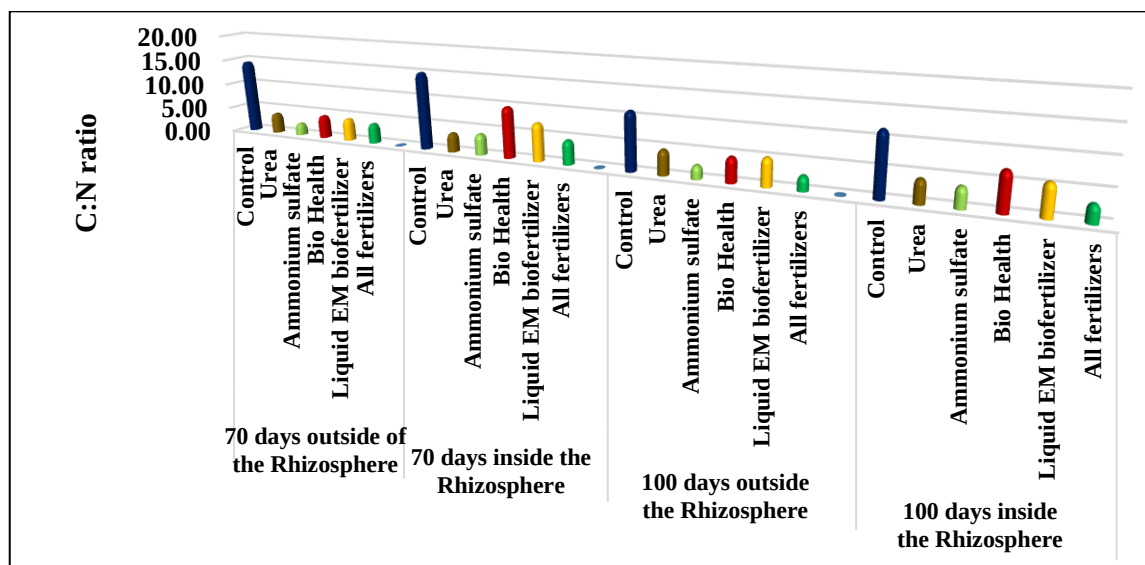


Figure 3. Impact of fertilizer type on the carbon-to-nitrogen ratio in the rhizosphere and bulk soils 70 and 100 days of planting (g kg^{-1}).

Conclusion

The study investigated the impact of biofertilizers and mineral fertilizers on soil nitrogen, organic carbon, and the nitrogen-to-carbon ratio. After 70 and 100 days, total nitrogen concentration in the rhizosphere and soils decreased. Biofertilizers and mineral fertilizers exceeded organic carbon concentrations in the rhizosphere and soils 70 days post-planting. Urea outperformed rhizosphere and bulk soils at 100 days. The rhizosphere had significantly less organic carbon than the soils in all treatments except the urea treatment as growth durations increased. Organic carbon levels increased in all rhizosphere treatments except the mixed fertilizer treatment. The findings showed that all treatments, except urea, had lower N:C ratios than the control as the growth period prolonged (100 days post-planting). The ratios varied depending on the carbon and nitrogen source.

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Effect of Spray Jasmonic Acid and Nano Iron on the Growth and Flowers of the Tecoma Plant, *Tecoma stans* L.

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Abstract. Due to the importance of cultivating ornamental trees The experiment was conducted at the research station of the Faculty of Agriculture - University of Kufa during the agricultural season 2024-2025 to demonstrate the role of Jasmonic acid and Nano iron in the growth and flowering of the Tecoma plant., by studying two factors: jasmonic acid at four concentrations (0, 15, 25 and 40) mg/L⁻¹ and the second factor was nano iron, at four concentrations (0, 30, 60 and 90) mg/L⁻¹. Both factors were sprayed three times, the first spray after transferring the seedlings to pots with a diameter of (26) cm and the second after 21 days from the first spray. The treatments were applied in a factorial experiment with two factors and three replications and the experiment was implemented using a complete randomized block design (R.C.B.D) 4×4×3 = 48. were analyzed using analysis of variance (ANOVA) and comparisons were made. According to the Least Significant Difference test (L.S.D) at a probability level (0.05). The results showed that spraying jasmonic acid at a concentration of 40 mg.L⁻¹ with nano iron at concentrations of 60 and 90 mg.L⁻¹. The best results that recorded a significant increase in plant height, number ofv leaves, number of days of crisis for flowering, number of floarets per inflorescence, leaf content of total chlorophyll, and total soluble carbohydrates. And carotene reached were in the treatment. The interaction was 40 mg.L⁻¹ jasmonic and 90 mg.L⁻¹ nano iron, which did not differ from the interaction of jasmonk at the same concentration with 60 mg.L⁻¹ nano iron. The mentioned interaction treatments differed significantly from the individual treatments and was also significantly different from the control treatment in all the studied parameters.

Keywords. *Tecoma stans* L, Jasmonic acid, Nano iron, Fertilization.

1. Introduction

Ornamental shrubs are important plants used to beautify and enhance public and private gardens, because of their size, pleasant fragrance, leaf and flower shape, crown shape, and growth habit [1]. One of the most famous of these shrubs is the Tecoma stans (yellow trumpet tree), which is one of the most important evergreen, fast-growing shrubs belonging to the Bignoniaceae family, of the genus Tecoma, which includes 14 species, including the Stans shrub, which is an ornamental shrub with an upright, branched stem with small branches, reaching a height of 2-4 meter [2]. the leaves are, green, pinnate, and serrated. Each leaf consists of 4-5 to lanceolate to

oblong leaflets. The flowers are bright yellow, funnel-shaped, slightly fragrant, and are borne in short clusters [3]. Its habitat is the Mediterranean Sea. Native to the tropical and subtropical regions of Central and South America and India[4].

The process of correct and balanced nutrition that provides the plant with all its nutritional needs, is important in increasing the rate of plant growth, completing the formation and improvement of flowers and their quality, and producing cut flowers with standard specifications, by spraying plant growth regulators because they play a major role in improving plant growth [5]. It is a organic compounds that encourage the absorption of nutrients and prepare carbon and energy. They are considered a very important factor in the integration of the stages of growth, metabolism and distribution within the plant. Among these hormones jasmonic acid (JA), is one of the plant hormones and one of the most important defense methods when exposed to stress, as it helps to mitigate the damage resulting from stress [6].

Nano-fertilizers are defined as substances with diameters are less than 100 nm[7]. They are in the form of micro or macro elements, or loaded into nanoparticles. They have proven their effectiveness compared to traditional fertilizers. this is due to the difference in the mechanism of action of these fertilizers in delivering nutrients to the plant, as well as increasing their efficiency[8]. Fertilizers, in addition to reducing nutrient loss, contribute to reducing environmental pollution, increasing the growth and productivity of agricultural crops, and reducing economic costs, as they have been proven to be a successful alternative to traditional chemical fertilizers [9].

Iron is an important element important and necessary elements for plant growth, as it affects many vital processes, through its direct participation as a structural part of plant. materials or through the activation of enzymatic processes within plant[10] .

Iron element is included important in the process of chlorophyll formation in plants, as it is a cofactor and activator of the reactions that form green pigments through a series of compounds, and these reactions end with the formation of chlorophyll molecule [11 and 12].

2. Materials and Methods

The experiment was carried out at the Agricultural research station of the College of Agriculture - University of Kufa, in a greenhouse covered with green netting, with a shade rate of 25% during the 2024-2025 agricultural season, to demonstrate the effect of jasmonic acid and Nano iron on the growth and flowering of the Tecoma plant. The seedlings were transferred on September 10, 2024, into plastic pots with a diameter of (25 cm) and a height of (28 cm) containing a culture medium consisting of soil and peat moss at a ratio of 1:3. Samples of the soil used in agriculture were analyzed and their chemical and physical properties and suitability. [13], Table(1).

Table 1. Physiochemical composition analyses of the potting soil.

The texture of potting soil (Sandy soil)	Profile	(%)
	Clay	16.28
	Silt	7.85
	Sand	74.18
Chemical analysis	Unit	Value
pH	-----	7.60
EC	Ds.M ⁻¹	3.70
N	mg.L ⁻¹	30.90
P	mg.L ⁻¹	12.30
K ⁺	mg.L ⁻¹	14.70
CaCO ₃	%	12.56

Orange matter (OM) % 2.20

Table 2. Physiochemical composition analyses of potting-peat moss.

Chemical analyses	Unit	Value
pH	-----	7.86
EC	Ds.M ⁻¹	2.69
N	mg.L ⁻¹	140
P	mg.L ⁻¹	160
K ⁺	mg.L ⁻¹	180
Mg ⁺⁺	mg.L ⁻¹	100
S	mg.L ⁻¹	120

The experimental treatments included two factors the first factor was spraying jasmonic acid at four concentrations (0, 15, 25 and 40) mg.L⁻¹, and the second factor was spraying nano iron at four concentrations: (0, 30, 60 and 90) mg.L⁻¹. with three replications as Complete Randomized Block Design (RCBD) the experiment data were collected and subjected to data analysis, where analysis of variance ANOVA was performed using the computing statistical program GenStat 12th Differences among the treatment's means were compared according to the least significant difference (L.S.D) at a probability level of 0.05. the first and second factors were sprayed (one month after transplanting the seedlings) (10/9/2024), and spraying continued every 21 days [14]. The spraying procedure was done three times for both factors; the first spray was applied after the when transplanting seedlings into pots while leaving 21 days between the first, second, and third spraythi plants were sprayed early in the morning until complete wetness using a hand sprayer (2 liters size). Also, a liquid detergent (two drops per liter) was added to the spray solution as a surfactant, taking into account the placement of a barrier between the treatments so as to avoid contamination between the experimental units. A layer of polyethylene was placed under the pots to prevent the growth of weeds in the lath house and to avoid the rise of salts in the experiment pots. Also, thin plastic mulch was placed beneath each pot to maintain moisture in the potting soil.

2.1. First: Vegetative Growth Indicators

2.1.1. Plant Height (cm)

Plant height was measured using a measuring tape from the soil surface of the pots to the highest point at the stage of flower bud emergence for each experimental unit, and the rate was calculated for each treatment.

2.1.2. Total Numbere of Leavese

The number of total leaves and each experimental unit were calculated, from which the average values for each treatment were calculated .

2.2. Second: Flower Growth Indicator

2.2.1. Number of Flower Inflorescences

The number of plant inflorescences for each experimental unit was calculated, and the average number of inflorescences for each treatment was extracted .

2.2.2. The Number of Florets in an Inflorescence

The number of florets per inflorescence was counted, and the average number of florets for the experimental units and each treatment was extracted.

2.3. Third: Chemical Indicators

- Estimation of the total chlorophyll pigment content of leaves ($100 \text{ mg} \cdot \text{g}^{-1}$) fresh weight.
- The chlorophyll pigment content of leaves was determined according to the method of [15]
- Estimation of the leaves' content of total soluble carbohydrates ($\text{mg} \cdot \text{g}^{-1}$)
- 2-The carbohydrate content of the leaves was estimated according to the method.[16]
- Estimation of the carotene pigment content of flowers ($\text{mg} \cdot 100 \text{ g}^{-1}$) is carried out according to the method [17].

3. Results

Results that spraying As show in Table (3) spraying with Jasmonic acid at a concentration of $40 \text{ mg} \cdot \text{L}^{-1}$ led to a significant increase in plant height, number of leaves, leaf content of total chlorophyll, leaf content of total soluble carbohydrates, and flower content of carotene pigment, as it reached 38.51 cm , $68.05 \text{ leaves plant}^{-1}$, $15.74 \text{ mg} \cdot 100 \text{ g}^{-1}$, $15.83 \text{ mg} \cdot \text{g}^{-1}$ and $13.39 \text{ mg} \cdot 100 \text{ g}^{-1}$ compared with the control treatment, which amounted to 33.76 cm , $59.01 \text{ leaves} \cdot \text{plant}^{-1}$, $14.37 \text{ mg} \cdot 100 \text{ g}^{-1}$, $14.91 \text{ mg} \cdot \text{g}^{-1}$ and $12.85 \text{ mg} \cdot 100 \text{ g}^{-1}$, respectively. We conclude from the results of Table (3) that spraying with jasmonic acid at a concentration of $40 \text{ mg} \cdot \text{L}^{-1}$ resulted in number of days of crisis for flowering Plant^{-1} , Number of florets in an inflorescence $^{-1}$ of the Tecoma plant, as it reached 192.44 days , $69.04 \text{ (floret}^{-1})$ compared with The comparison treatment amounted to 195.16 days and $60.06 \text{ (floret}^{-1})$ respectively.

The results of the same tables also showed that spraying Nano iron on the Tecoma plant a concentration of $90 \text{ mg} \cdot \text{L}^{-1}$ led to a significant increase in the height of the plant, the number of leaves, the leaf content of total chlorophyll, the leaf content of total soluble carbohydrates, and the flower content of carotene pigment, as it reached 37.16 cm , $66.04 \text{ leaves} \cdot \text{Plant}^{-1}$, $15.79 \text{ mg} \cdot 100 \text{ g}^{-1}$, $15.85 \text{ mg} \cdot \text{g}^{-1}$, $13.16 \text{ mg} \cdot 100 \text{ g}^{-1}$ compared to the measurement treatment, which amount ed to 34.30 cm , $58.02 \text{ leaf} \cdot \text{plant}^{-1}$, $14.19 \text{ mg} \cdot 100 \text{ g}^{-1}$, $15.85 \text{ mg} \cdot \text{g}^{-1}$, $13.45 \text{ mg} \cdot 100 \text{ g}^{-1}$ sequentially.

It is noted from the results of Tables (3) that spraying Nano iron at a concentration of $90 \text{ mg} \cdot \text{L}^{-1}$ on the Tecoma plant led to a significant increase in the number of days of crisis for flowering Plant^{-1} , the Number of florets in an inflorescence (floret $^{-1}$) inflorescence, as it reached 192.91 days , $68.09 \text{ (floret}^{-1})$ compared to the measurement treatment, which amounted to $3.84 \text{ inflorescences}^{-1}$ and $7.08 \text{ inflorescences}^{-1}$, respectively.

The results of Table (3) show that spraying with jasmonic acid at a concentration of $40 \text{ mg} \cdot \text{L}^{-1}$ and Nano iron at a concentration of $90 \text{ mg} \cdot \text{L}^{-1}$ led to a significant increase in plant height, number of leaves, total chlorophyll content of leaves, total soluble carbohydrates, and flower content of carotene oide pigment reached 42.36 cm , $78.03 \text{ leaf} \cdot \text{plant}^{-1}$, $18.38 \text{ mg} \cdot 100 \text{ g}^{-1}$, $16.80 \text{ mg} \cdot \text{g}^{-1}$, $13.93 \text{ mg} \cdot 100 \text{ g}^{-1}$. It follows (3) that spraying with Jasmonic acid at a concentration of $40 \text{ mg} \cdot \text{L}^{-1}$ with iron The nano iron at a concentration of $90 \text{ mg} \cdot \text{L}^{-1}$ had a significant effect on the number of inflorescences, reaching 188.80 days and $66.04 \text{ (floret}^{-1})$, compared to the spraying treatment with distilled water, which amounted to 199.27 days and $45.00 \text{ (floret}^{-1})$, respectively.

Table 3. Response of vegetative growth and flowering in *Tecoma stans* L. to spraying with jasmonic acid and Nano iron.

Jasmonic acid (JA) mg.L ⁻¹	Nano iron mg.L ⁻¹	Leaf content of macroelements (gm ⁻¹)			Days to first flower spike	Number of pink inflorescences . Plant ⁻¹	Plant height(cm)	Number of papers leaf.plant ¹
		Chl	CH O	Carotene				
0	0	12.67	12.88	11.79	199.27	45.00	25.44	45.05
	30	13.51	13.95	12.13	195.87	54.01	31.81	53.02
	60	14.96	16.22	13.34	194.17	68.00	37.71	65.01
	90	16.35	16.59	14.16	191.33	75.04	40.08	72.07
15	0	16.04	15.84	12.73	193.30	61.06	36.32	66.04
	30	15.23	14.18	12.94	193.63	63.05	30.67	63.04
	60	13.60	13.47	12.12	196.97	51.03	29.98	49.08
	90	14.76	16.36	13.52	193.50	70.00	36.29	66.06
25	0	14.71	14.96	13.02	194.63	63.04	39.17	62.07
	30	16.56	16.54	13.77	192.23	67.05	36.33	70.00
	60	16.67	15.51	13.18	190.73	73.09	29.78	72.05
	90	13.69	13.63	12.19	198.00	48.03	29.90	48.01
40	0	13.33	14.24	12.62	195.13	60.00	36.25	58.04
	30	15.01	15.93	13.01	194.63	66.08	39.13	64.01
	60	16.23	16.33	14.00	191.20	68.07	36.32	73.03
	90	18.38	12.88	13.93	199.27	45.00	42.36	78.03
L.S.D. (P≤0.05)	Jasmonic acid	2.010	1.612	1.197	3.922	15.23	4.236	12.18
	Nano iron	2.010	1.612	1.197	3.922	15.23	4.236	12.18
	Interaction	4.021	3.224	2.394	7.843	30.46	8.472	24.35

4. Discussion

It was noted from the results of Table (3) that there was a significant increase in vegetative and chemical growth indicators. This is due to the role of jasmonic acid in regulating vital processes in plants, including carbon metabolism, as it was a primary source of nitrogen necessary for

building proteins and most of the enzymes involved in building plant cells Stimulating cell division and elongation and regulating physiological processes, which in turn leads to an increase in plant height and an increase in the number of leaves[18]. The reason for these significant effects may be attributed to the role of Jasmonic acid in increase the stimulation of the growth regulator cytokinin and subsequently increasing plant pigments thus leading to an increasing in the total chlorophyll content of leaves. In addition, it controls ethylene production in plant tissues, causing reduced chlorophyll decomposition in leaves [19]. This may be due to the physiological role of methyl jasmonic acid, which works to mitigate the harmful effects of water deficiency and increase the hormone ABS in the leaves, in addition to increasing the duration of stomatal closure, thus reducing the rate of transpiration, increasing the concentration of CO₂, and increasing the percentage of protein, in addition to protecting the cell membrane, thus increasing the efficiency of carbon metabolism and carbohydrate synthesis. This is reflected in the chemical indicators of plants. This result is identical to that reported [20].

The results of Table (3) indicate that spraying with jasmonic acid led to a significant increase in flower growth indicators. This may be due to the role of jasmonic acid in increasing the activity of the carbon assimilation process, which acts as a stimulant for plant growth in the structural unit in the formation of proteins that help stimulate enzymes that have a role in cell division at the plant apex and the formation of vegetative buds, leading to a change in the shape and size of the apex and turning it into floral apices instead of vegetative buds, to become places with metabolic activities to attract carbohydrates manufactured in these flower buds for the purpose of distinguishing the emergence of floral inflorescences, the effects of Jasmonic acid on vegetative growth indicators may be accumulated in increasing vegetative growth rates, including plant height, which leads to an increase in the number of floral inflorescences and an increase in the number of florets in the floral inflorescence [21].

the results in Table (3) that spraying with iron led to an improvement in vegetative and chemical growth indicators. In plants iron plays a vital role as an auxiliary agent in the formation of chlorophyll, which is necessary for photosynthesis and energy production. Iron is also involved in respiration and the reduction of nitrates and sulfates[22]. and in the formation of enzymes necessary for plant growth and development, and helps to resist oxidative stress, This may be due to the fact that nano iron is an important and necessary element for plant nutrition, and that many carbohydrate metabolism enzymes need iron as a basic catalyst for their work [23]. It is also involved in regulating the osmotic potential in the system's guard cells. The stomatal system activates approximately 120 enzymes that are involved in Calvin cycle reactions and nucleic acid metabolism, This may be due to the important role of iron in plant metabolic processes, such as the process of photosynthesis and the construction of carbohydrates, as it is an essential and effective element in a large number of plant enzymatic systems and the citric acid cycle are necessary in the respiration process. It also plays a major role in the transport and distribution of starch[24]. Iron is considered one of the necessary and important elements for plant nutrition. It has been noted that many carbohydrate metabolism enzymes need iron as a catalyst for their work and that this has a significant effect. In increasing chemical indicators [25]. All of these roles are reflected positively in vegetative growth indicators and thus lead to giving the best results in chemical growth indicators. This result is consistent with the findings of Alam when spraying iron on the caldiolus plant. It is consistent with and[26], as shown by the results of Tables (3) for spraying nano element iron. Tecoma plants, led to a significant increase in floral indicators. This may be due to the role of iron, which is an essential element in the formation of the chlorophyll molecule, as it plays an important role in the process of photosynthesis and stimulates formation of chlorophyll. It interacts with many vital enzymes in plants, thereby increasing their activity and effectiveness. It is considered an essential responent of respiratory enzymes, metabolism, and the

protein synthesis process, which positively reflects vegetative growth indicators and thus increases the number of leaves, which positively reflects the characteristics of flowering growth [27].

Conclusions

Results showed that spraying the the Tecoma plant with the growth regulator jasmonic acid and nano iron revealed a significant response of the plant in all growth and flowering indicators under study. Further, spraying nano iron with high concentration had a positive effect among the other concentrations, which showed the best results in most of the indicators. Also, the plants were characterized by giving the highest mean in vegetative, flowering, and chemical indicators when sprayed with the growth regulator Jasmonic acid at 40 mg. L⁻¹ with nano iron at a concentration or 90 mg. L⁻¹.

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Assessment of Accumulation of Heavy Metals in Date Palm Trees Under the Influence of LukOil Facilities in Southern Iraq

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Abstract. Heavy metals are among the pollutants that have become a global problem because of their environmental stability. The entry of these elements into the food chain is a serious threat to humans and other organisms, and seems to be a more serious threat to agricultural lands around industrial areas. The purpose of this study was to evaluate the accumulation of heavy metals in dust deposited on the leaves of palm trees and in the tissues of these leaves under the influence of burning oil flares in southern Iraq. Date palm leaves were collected from 35 location around oil flares located in Basra province. After separating the dust on the leaves, both date leaf samples and dust samples were taken to the laboratory to measure the total concentration of Pb, Ni, Fe, Zn, Cu and Mn. Pollution indices including pollution factor index is another index for evaluating the level of soil pollution (CF), in the study area. The results of measuring the concentration of heavy metals in dust showed that the average concentration of iron, zinc, copper, manganese, nickel, and lead in dust was 1375, 110, 23.52, 718.3, 99.51, and 48.59 mg/kg, respectively, and 150.5, 116.1, 3.98, 20.47, zero and zero mg/kg in the leaves of trees. In fact, the concentration of the two heavy metals nickel and lead in the leaves of the trees was lower than the detection limit of the device. The contamination factor (CF) showed very high pollution for lead, no pollution for manganese, and low to moderate pollution for Zn, Cu, Ni and Fe. In general, the highest concentration of heavy metals is observed in the southern areas where there is a concentration of flares no. 3, 4 and 5, and the amount of pollution decreases by moving towards the northern areas. Around the towers flares no. 1 and 2, which are far from each other, the contamination with heavy elements under study is more than that in other parts of the region. It seems that the oil flares that have been burning oil gases since about 12 years ago (from 2010) and releasing thier residues into the air and the environment in general are the source of pollution in the region.

Keywords. Heavy Metals, Burning Flares, Petroleum Gases, Basra Province, Dust, Pollution.

1. Introduction

Environmental pollution by heavy metals causes undesirable changes in the physical, chemical, and biological properties of the primary resources of life, namely water, air, and soil; threatening the survival and health of humans and other organisms, and restricting their activities. Although heavy metals have various sources, they mainly enter the environment through the atmosphere [1].

Heavy metals are defined as metals with a density greater than 5 g per cubic centimeter, their major sources are industrial activity, mining, fuel combustion, automobile transportation, and the use of pesticides and fertilizers in agriculture [2].

Research has shown a significant correlation between the amount of suspended particulate matter deposited in the air and its concentration in the leaves of various tree species, grasses, and ornamental plants [3]. Among the plants used for biomonitoring, trees can indicate soil pollution owing to their long lifespan. Evergreen plants, better reveal changes in air pollution than deciduous plants. Some researchers have indicated that a hyperaccumulator plant of heavy metal is identified by four specific indicators: a) the ability to accumulate metals in the aerial part, meaning the threshold level of metal in the aerial part must be higher than that of normal plants, b) having a bioconcentration factor greater than one, c) having a transfer factor greater than one, and d) the ability to tolerate high metal concentrations. Given the risks posed by pollutants to living organisms, sufficient information regarding the type and amount of pollutants in urban environments is of great importance [4].

Addressing the issue of heavy metals is particularly important because of their toxicity, bioaccumulation, and long-term persistence in living organisms. These metallic elements can be classified into two categories: essential metals, such as Cr, Co, Zn, Cu, Mn, Fe, and non-essential metals such as lead, cadmium, mercury, and arsenic [5]. Heavy metals typically precipitate in surface soils, are absorbed by the soil, and can lead to farmland contamination and degradation of natural resource areas, ultimately entering the soil-plant-animal-human cycle [6].

The most important issue concerning heavy metals is their non-metabolize in the body. In fact, once heavy metals enter the human body, they are not excreted, but rather deposit and accumulate in tissues such as fat, muscles, bones, and joints, which leads to various diseases and complications in the body. The presence of heavy metals above defined standards in the environment causes environmental problems for the residents of that location and the ecosystem. The effects of heavy metals on humans vary, with the most significant being the emergence of neurological disorders. However, the toxic properties and bioaccumulation potential of heavy metals in plants and animals, as well as their entry into the food chain, double the risks associated with them and create significant ecological impacts [7].

Basra is situated in a fertile area that has facilitated agricultural prosperity in this province. Products such as dates, wheat, barley, corn, tomatoes, and vegetables are cultivated in this province. Additionally, livestock, beekeeping, and fishing flourish in Basra. Historically, this city was the main center of date cultivation and production in the world [9].

The aim of this study was to determine the concentration of heavy metals such as lead, nickel, manganese, iron, zinc, and copper present in dust settled on the leaves and inside the tissue of date palm leaves affected by the activities of oil rigs burning oil gases around Basra, located in southern Iraq. In addition, accumulation, and pollution load indices were used to determine the dust pollution around oil rigs located in southern Iraq.

2. Materials and Methods

2.1. Study Area

In Basra Province, various industries operate that result in environmental pollution due to compounds such as oil, and these compounds are significant factors in the increased levels of certain heavy metals in the environment. Considering the above, studying the behavior of plants in areas contaminated with heavy metals and avoiding their use in these regions owing to serious transmission to humans is very important. The area of Basra province is 19070 square kilometers, and the population of this province in 2003 was 2,600,000 and including many oil activities in different locations, such as the Majnoon field (fig.1). The largest oil field in Iraq, is located in the south of the country, near the city of Basra [8].

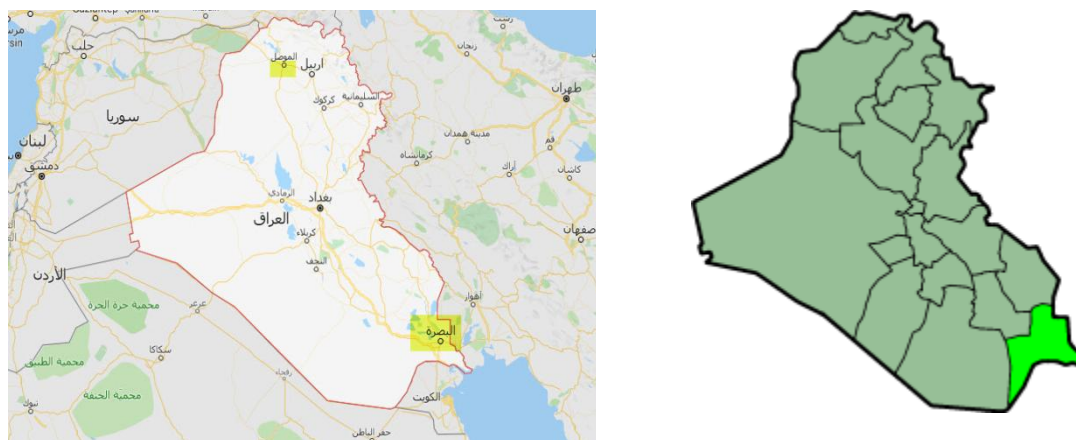


Figure 1. Shows the position of Basra Province on the map of Iraq and the region.

In the study area, there are five flaring towers (about 8-9 km the distance between the towers) that continuously burn gas emissions from oil wellheads (30 m the height of towers), releasing soot and pollutants resulting from the combustion of these gases into the environment of the region. The tower located to the west of Area 1 is named tower number 1, the tower located to the east is named tower number 2, and the three towers to the southwest of the area are referred to as tower numbers 3, 4, and 5.

2.2. Sampling

A total of 35 date palm trees around the gas flaring were used to collect leaf samples to measure heavy metals. Fig. 2 illustrates the studied area along with the locations of the oil towers and points for collecting leaves from date palm trees in the region.

Leaf samples were collected from all four sides of each tree, and leaves from each tree were collected in a separate envelope. In total, 35 envelopes containing leaf samples from the trees in the region were prepared. Care was taken during leaf collection to avoid dust from leaves. Leaves were collected from the dust present on them. Figure 2 shows the collection of date palm leaf samples by the student along with the thesis advisor. After the leaves were collected, the dust present was initially brushed off and placed in separate envelopes, which were then transferred to the laboratory for heavy metal analysis.

2.3. Laboratory Analyses

The leaf samples from which dust was removed were ground and dried in an oven at 70 °C for 48 h. The digestion of plant samples was carried out by adding 5 ml of 65% concentrated nitric acid to 2 g of ground date palm leaves. After heating to 110 °C for 2 h, the samples were cooled to room temperature. Then, 1 ml of 30% hydrogen peroxide was added to each sample, heated at 70 °C, and the solution was filtered through filter paper, brought to a volume of 50 ml, and the concentration of heavy metals including iron, zinc, copper, manganese, nickel, and lead were read measured using an atomic absorption (novAA.800 / Analytik Jena) device [10].



Figure 2. Sampling of leaves and dust on palm tree leaves in the study area

To measure the concentration of heavy metals in the dust collected from the leaves, the same method as that used for the soil was applied. For this purpose, one gram of dust was placed in a 150 ml Erlenmeyer flask, and 5 ml of acid mixture (2:1 nitric acid, hydrochloric acid) was added. After a day, the sample was placed on a heater for 15 minutes at 60 °C, and the temperature was raised to 120 °C and maintained for 75 min., or until the sample became colorless. The clear solution was then filtered and brought to a volume of 50 ml, and the concentration of each element was read using an atomic absorption device [11].

2.4. Pollution Factor Index (CF)

The pollution factor index is another index for evaluating the level of soil pollution based on Equation 1, and is equal to the pollution index (PI).

$$PI=CF = C_n/B_n \quad (1)$$

Where C_n is the concentration measured for each heavy element in the sample and B_n is the concentration measured for the same element in the control sample, which in this study was taken as the average concentration of metals at stations 21, 22, and 23 as the geochemical control [12].

3. Results

Descriptive statistics of heavy metal concentrations in date palm leaf samples are presented in Table 1 and in dust samples from the leaves are presented in Table 2, respectively. The elements under investigation were, zinc, copper, manganese, nickel, and lead.

3.1. Concentration of Heavy Metals in Leaves

The results of measuring heavy metal concentrations in the leaves of date palm trees show that the average concentrations of iron, zinc, copper, and manganese were 150.5, 116.1, 3.98, and 20.47 milligrams per kilogram of dry matter, respectively, while the highest concentrations of these elements were 194.6, 156.2, 5.53, and 30.04 milligrams per kilogram of dry matter, and the lowest concentrations were respectively measured at 85.23, 67.28, 2.06, and 8.19 milligrams per kilogram of dry matter in the leaves. Among the elements in the leaf tissues of date palm, the highest concentrations pertained to Fe and Zn. It seems that the high concentration of elements is due to the plant's need for all four heavy elements that are absorbed through the plant roots from the soil and reach the leaves of the plant. However, some of these elements present in the leaf tissues of trees likely entered the environment from the burning of oil rigs.

The presence of these elements along with nickel and lead in the dust of the area indicates that at least part of these elements has an origin from burning fossil fuels that enter the environment from the local oil rigs. It is worth noting that the concentrations of lead and nickel in the leaf tissues of date palm trees were below the detection limit of the device. The concentrations of the two heavy metals nickel and lead were below the detection limit of the device. [13] reported that the results of assessing heavy metal pollution in the leaves of date palm trees located in Basra Province, Iraq, showed that the highest level of pollution was observed in this area, with concentrations in soil and leaf samples being 20.58 and 19.50 mg/kg, respectively. Additionally, the concentration of lead was recorded at 40.40 and 0.115 mg/kg in the soil and leaves of the date palm tree.

[14] stated that plants are collectors of all air pollutants, and their chemical composition may serve as a good indicator for polluted areas. Among the heavy metals studied by these researchers, iron, zinc, copper, and manganese are considered essential micronutrients required by plants. [15] conducted a study on the toxicity of some heavy metals in plants in Baghdad, Iraq, and reported the average concentrations of iron, nickel, and lead in date palm leaves as 211, 45, and 30 mg/kg dry matter, respectively. They also mentioned that heavy metals tend to accumulate more in the leaves of plants than in the fruits, with the average concentrations of these three elements in date fruits measuring 138, 47, and 31 mg/kg dry matter, respectively.

Many researchers studying water, soil, and plants in Baghdad have detected a high concentration of heavy metals in these environments [16].

The results in table (2) showed that the average concentrations of lead, iron, zinc, copper, manganese, and nickel were found to be 59.48, 1375, 110, 52.23, 3.718, and 51.99 milligrams per kilogram of dust, respectively, while the highest concentrations of these elements were 64.12, 1796, 182.3, 46.16, 904.9, and 144.1 milligrams per kilogram and the lowest concentrations were measured at 26.71, 501.4, 73.86, 14.26, 488.6, and 58.04 milligrams per kilogram, respectively. The highest concentrations pertained to the elements iron, manganese, and zinc. It seems that burning petroleum materials has a high concentration of these heavy elements. Although the elements nickel, lead, and copper are also present in the dust.

[17] in their research on agricultural soils and dust present in the gas industry in the Kurdistan Region of Iraq found that the burning of gas plant by-products contributes to the increase of heavy metals. The findings of [18] indicated that the heavy metal lead in the dust of Tehran city

has severe enrichment in both warm and cold seasons, and their investigations suggested that the source of heavy metals is anthropogenic.

Table 1. Descriptive statistics of the concentration of heavy metals studied in date palm leaves.

Variable	Milligrams per kilogram ppm					
	Ni	Mn	Cu	Zn	Fe	Pb
Sample Size	12	12	12	12	12	12
Mean	0	20.47	3.98	116.1	150.5	0
Maximum	0	30.04	5.53	156.2	194.6	0
Minimum	0	8.19	2.06	67.28	85.23	0
Standard Deviation	0	8.12	1.08	32.18	39.39	0
Coefficient of Variation	0	64.39	15.27	27.71	26.18	0

Table 2. Descriptive statistics of measured heavy metal concentration studied in dust on leaves.

Variable	(milligrams per kilogram ppm)					
	Ni	Mn	Cu	Zn	Fe	Pb
Sample Size	12	12	12	12	12	12
Mean	51.99	3.718	52.23	110	1375	59.48
Maximum	144.1	904.9	46.16	182.3	1796	64.12
Minimum	58.04	488.6	14.26	73.86	501.4	26.71
Standard Deviation	31.82	125	34.8	30.2	468.9	67.12
Coefficient of Variation	98.31	17.4	43.35	46.27	34.11	26.05

Table 3. Comparison of the average concentration (mg/kg) of heavy metals in the treatments studied in the leaf tissue of the date palm tree.

Heavy Metal / Care	Fe	Pb	Cu	Ni	Zn	Mn
Trees around Tower 1	152.40b	0.00	4.87a	0.00	151.02a	16.03b
Trees around Tower 2	179.59a	0.00	4.36a	0.00	121.62b	27.68a
Trees around Tower 3,4,5	179.11a	0.00	4.30a	0.00	116.87ab	27.89a
Trees far from oil towers (control)	90.62c	0.00	4.38b	0.00	76.42c	10.34c
Significance Level _{0.05}	**	**	**	**	**	**

In Table 3, the results showed a comparison of the average concentration (mg/kg) of heavy metals in the studied treatments (in the leaves of date palm trees close to 5 oil rigs compared to trees far from oil rigs as a control) is presented, and in Table 4-4, a comparison of the average concentration (mg/kg) of heavy metals present in the dust settled on the leaves of date palm trees, which is significant at the 0.01 (**) and 0.05 (*) level, is provided.

Table 4. Comparison of average concentrations (mg/kg) of heavy metals present in dust on tree leaves.

Treatment / Heavy Metal	Fe	Pb	Cu	Ni	Zn	Mn
Trees around rig 1	1440.53b	53.14ab	23.61ab	88.27bc	104.87b	801.14ab
Trees around rig 2	1721.80a	58.67a	24.28ab	115.89ab	116.63b	719.59b
Trees around rigs 3, 4 and 5	1697.44a	51.29a	34.95a	138.89a	156.66a	851.89a
Trees away from oil rigs (control)	631.25c	28.80c	14.82c	62.64c	75.03c	544.52c
Significance level _{0.05}	**	**	**	**	**	**

The comparison of average data for each heavy metal in samples of date palm fronds and in the dust present on the leaves of the trees is presented separately in Figures 1 to 10.

As shown in fig. 3, the average amount of iron measured in the date palm leaves differed among the treatments studied compared to the control, and the difference was significant at the 0.001 level. However, no significant difference observed between Treatments 2 and 3. Treatment 1 included trees around Tower 1. Treatment 2 included trees around tower 2. Treatment 3 included trees around oil towers 3, 4, and 5, and Treatment 4 included trees far from the oil towers (control).

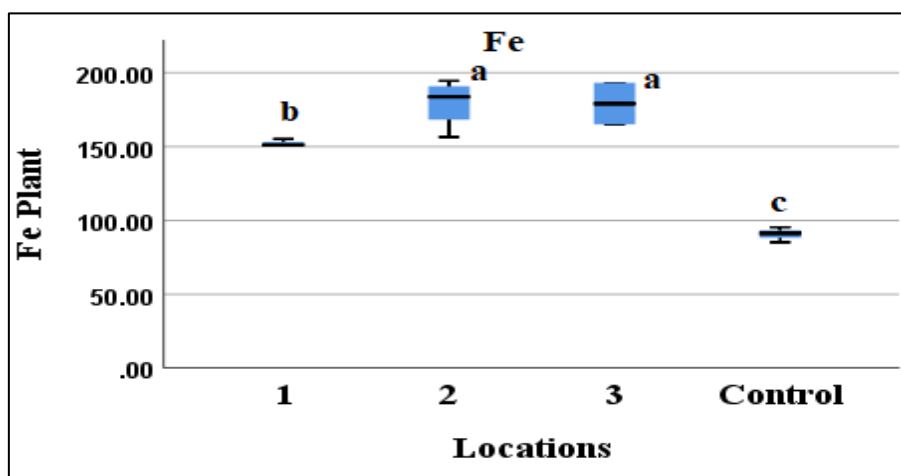


Figure 3. Comparison of the average iron content in date palm leaves in the study area (mg/kg).

Fig. 4, it shows that the average amount of iron measured in dust on date palms differs between the treatments studied and the control, with this difference being significant at the 0.001 level. However, no significant difference were observed between Treatments 2 and 3.

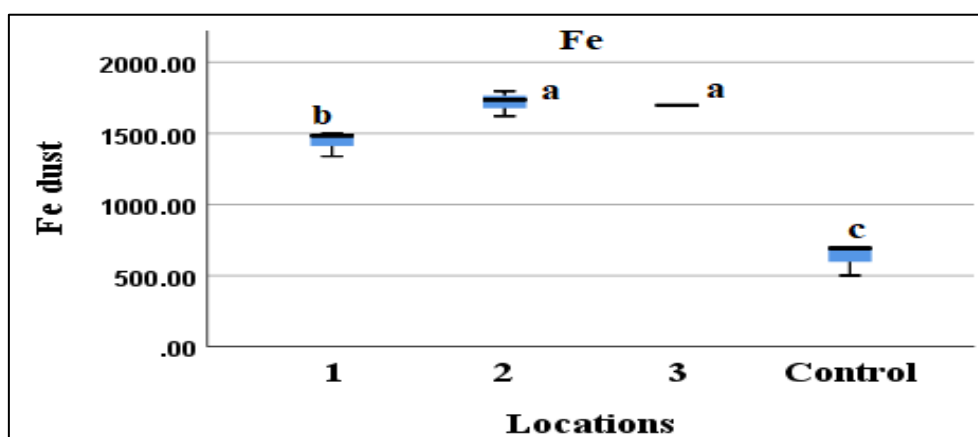


Figure 4. Comparison of the average iron content in the dust of the studied area (milligrams per kilogram).

Fig. 5, it shows that the average amount of zinc measured in leaves on date palm tree differs between the studied treatments and the control, and their difference is significant at the 0.001 level.

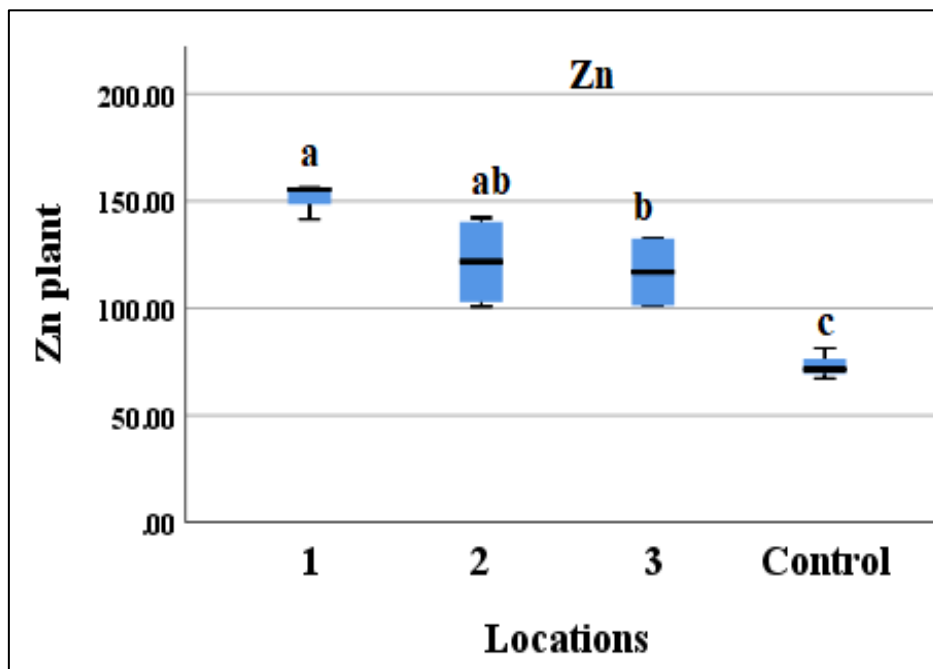


Figure 5. Comparison of the average zinc content in date palm leaves in the study area (mg/kg).

Fig. 6, shows that the average amount of zinc measured in dust differed between the studied treatments and the control, and the difference was significant at the 0.001 level. However, no significant differences were observed between Treatments 1 and 2.

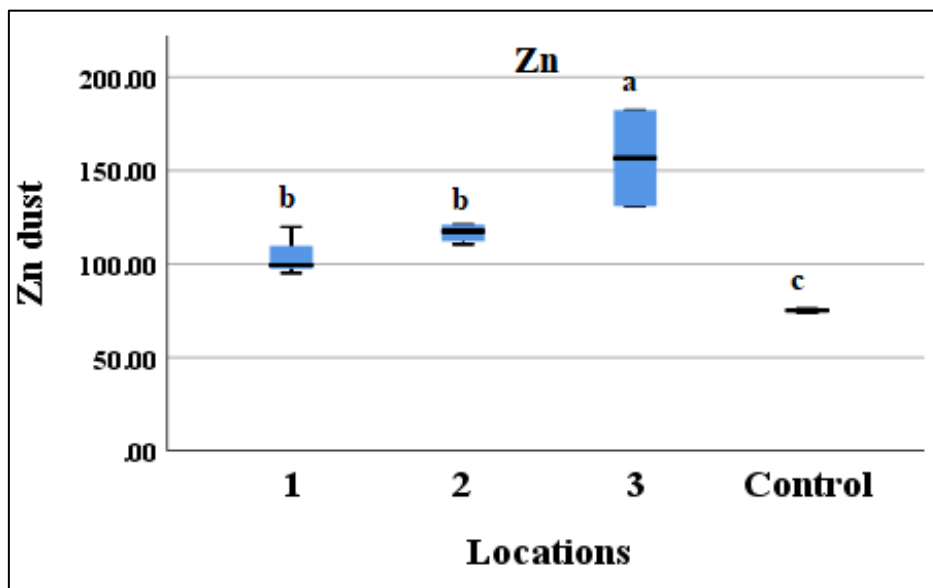


Figure 6. Comparison of the average zinc content in dust in the study area (mg/kg).

Fig. 7, shows that the average amount of copper measured in date palm leaves differed between the studied treatments and the control, and the difference was significant at the 0.001 level. However, no significant difference was observed among treatments 1, 2, and 3 respectively.

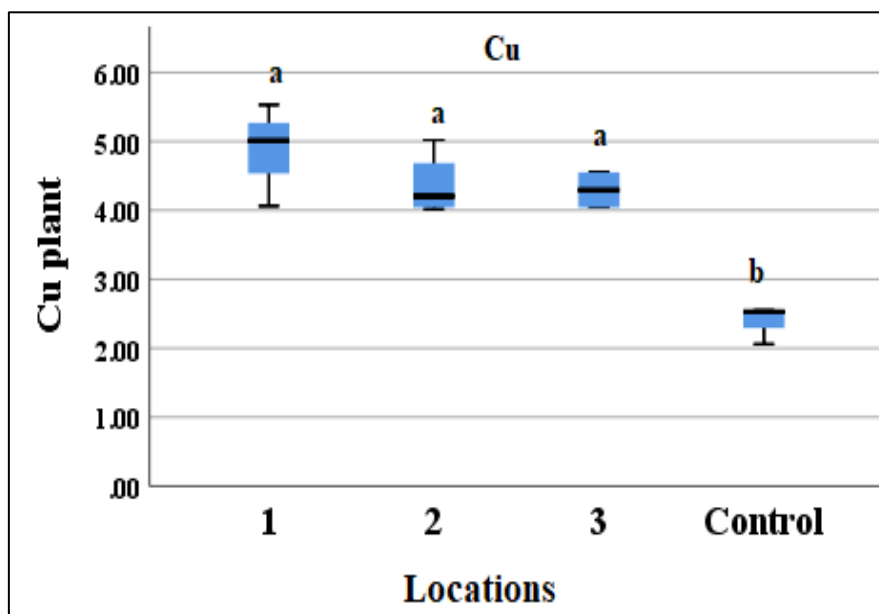


Figure 7. Comparison of the average copper content in date palm leaves in the study area (mg/kg).

Fig. 8, shows that the average amount of copper measured in dust differed between the studied treatments and the control, and the difference was significant at the 0.001 level. However, no significant differences were observed among treatments 1 and 2.

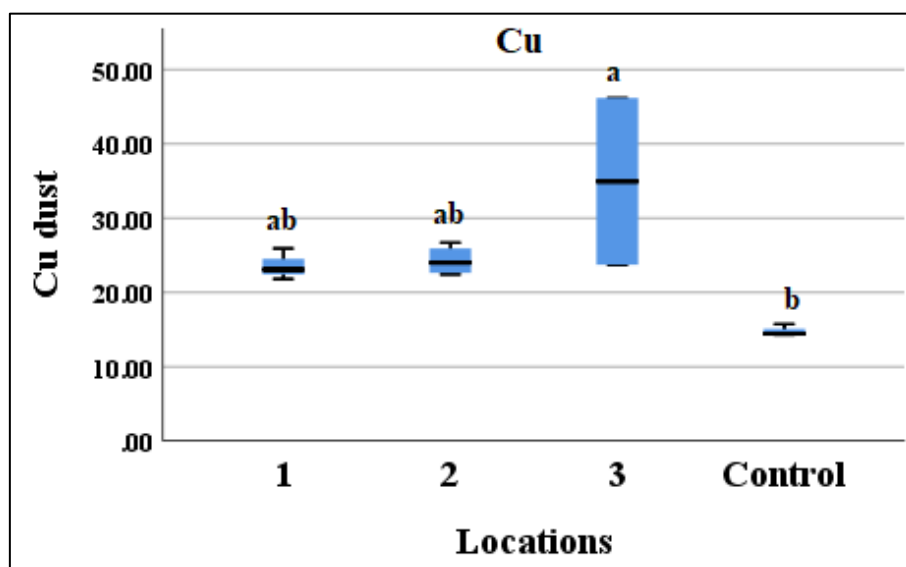


Figure 8. Comparison of the average amount of copper in dust in the study area (mg/kg).

Fig. 9, shows that the average Mn content measured in date palm leaves differed between the studied treatments and the control, and the difference was significant at the 0.001 level. However, no significant differences were observed among treatments 2 and 3.

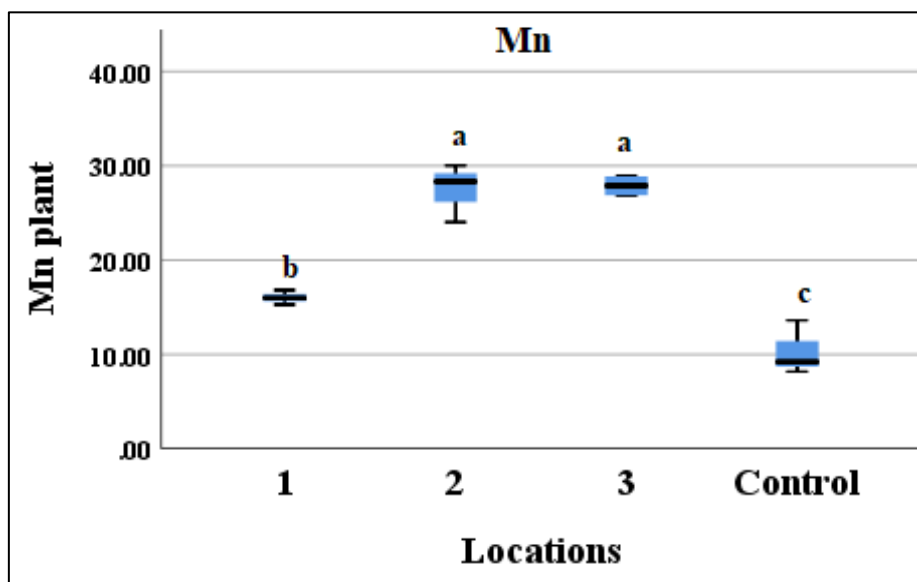


Figure 9. Comparison of the average manganese content in date palm leaves in the study area (mg/kg).

As shown in Figure (10) the average amount of Mn measured in the dust differed between the studied treatments and the control, and the difference was significant at the 0.001 level.

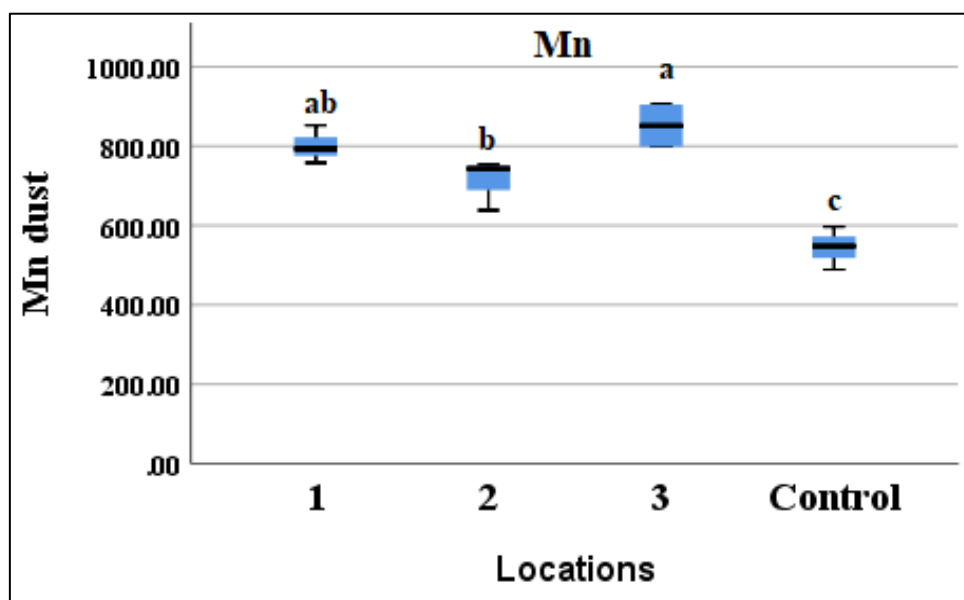


Figure 10. Comparison of the average amount of manganese in dust in the study area (mg/kg).

Fig. 11, shows that the average amount of Ni measured in the dust on leaves differed between the studied treatments and the control, and the difference was significant at the 0.001 level.

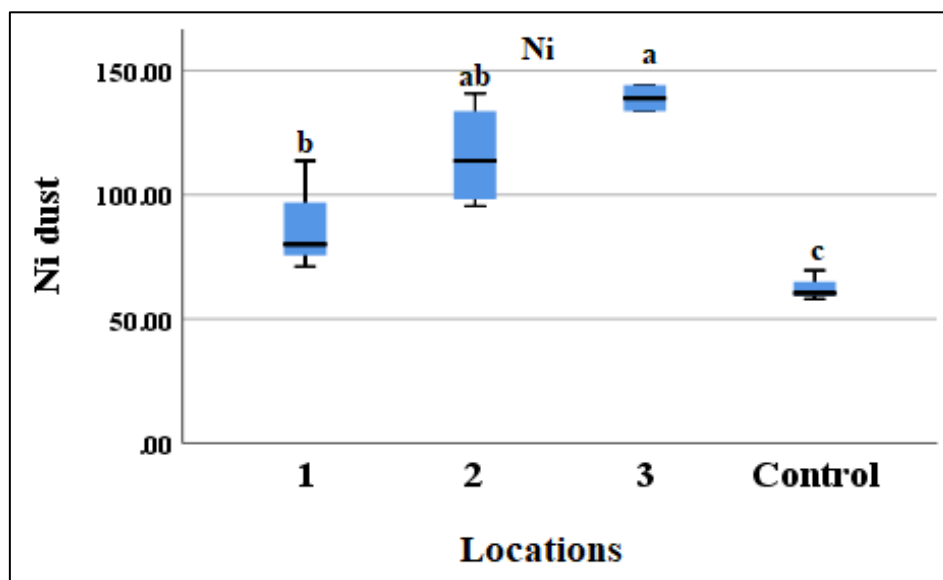


Figure 11. Comparison of the average nickel content in dust in the study area (mg/kg).

Fig. 12, shows that the average amount of Pb measured in the dust on tree leaves differed between the studied treatments and the control, and their difference were significant at the 0.001 level.

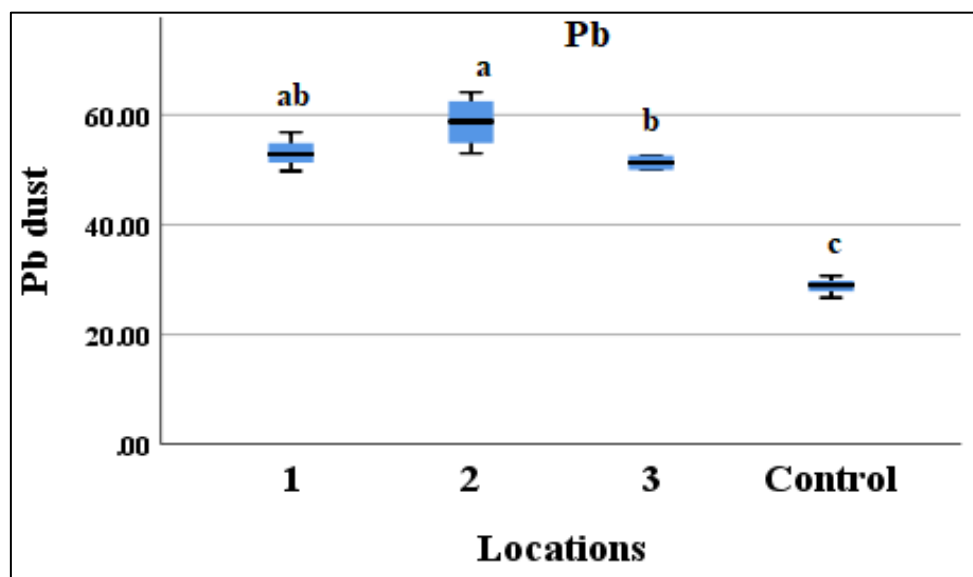


Figure 12. Comparison of the average lead content in dust in the study area (mg/kg).

3.2. Pollution Factor (CF)

The maximum, minimum and average pollution indices of the studied heavy metals are listed in Table 5. In this study, soil pollution indicators, including the contamination factor (CF) indicated very high pollution for Pb, no pollution for Mn, and low to moderate pollution for Zn, Cu, Ni, and Fe respectively.

Studies on the environmental pollution intensity of sediments in the Shiroud River using the geo-accumulation index and enrichment factor showed that this area was classified from low to severe pollution for Pb and Zn elements. Other researchers have also utilized the geo-accumulation index and enrichment factor to investigate and identify pollution sources, reporting that the most significant sources of pollution for Zn, Pb, and Cd are mining, industrial, and transportation activities [19].

Table 5. Maximum, minimum and average pollution index in the studied heavy metals

	Pb	Fe	Zn	Cu	Mn	Ni
Minimum	7.95 (22 Station)	0.32 (21 Station)	0.71 (21 Station)	0.96 (32 Station)	0.75 (21 Station)	0.88 (30 Station)
Minimum	19.8 (18 Station)	1.14 (16 Station)	1.75 (8 Station)	3.10 (8Station n)	1.43 (29 Station)	2.31 (6 Station)
Average	13.55	0.82	0.94	1.38	1.08	1.48

The results in table (6), shows the different classes of contamination factor (CF) in the study. Studies of soil pollution in northern Iraq indicate that industrial activities, the combustion of fossil fuels, and the extraction of nickel, along with other anthropogenic waste including the use of chemical fertilizers in agriculture, especially ammonium phosphate, are primary sources of soil pollution with cadmium and nickel [20].

Studies on pollution in industrial areas surrounding Wasit Province in Iraq indicated that pollution indices were average for all metals, except for Cd, which showed a significant increase [21].

Table 6. Standard classification of pollution factors [22].

Pollution level	Class	CF
Low pollution	1	$1 \leq CF$
Moderate pollution	2	$3 \leq CF$ < 1
Severe pollution	3	$6 \leq CF$ < 3
Very severe pollution	4	$4 > CF$

Conclusion

- The highest concentration of heavy elements in the dust of the area is related to Fe, Mn, and Zn. Although Ni, Pb, and Cu are also present in the dust. It seems that the combusted oil materials have a high concentration of these heavy elements.
- The highest concentration of elements within the tissues of date palm leaves was related to Fe, Zn, Cu, and Mn. The concentration of Pb and Ni in leaves was below the detection limit of the measuring device. The Pb enrichment level in some sampling points is severe, while in other points it is very severe, but the enrichment of other heavy elements in most points is low and in some points is moderate.
- The pollution factor index (CF) for Pb showed very high pollution, while for Mn it showed no pollution, and for Zn, Cu, Ni, and Fe, it indicated low to moderate pollution respectively.

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Assessment of the Accumulation of Heavy Metals in Soils Around Lukoil Company in Southern Iraq

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Abstract. The entry of heavy metals into the food chain is a serious threat to humans and other organisms, and seems to be a more serious threat to agricultural lands located around industrial areas. The purpose of this study was to evaluate the accumulation of heavy metals in the soils of areas around burning oil flares in southern Iraq. For this purpose, 35 points were selected on the land around the oil flares located in Basra Province. Sampling was done from a 0 to 15 cm layer of soil and samples were taken to the laboratory to measure the total concentration of heavy metals including Pb, Ni, Fe, Zn, Cu and Mn. The results of measuring the concentration of heavy metals in the soil showed the average of iron, zinc, copper, manganese, lead and nickel as 1370, 102.6, 19.9, 673.1, 45.1 and 96.9 mg kg⁻¹, respectively. Analysis of the variance of the means of heavy elements showed that there is a significant difference between the heavy elements in the soils around the flares and the control soils that are far from the flares. The concentrations of these elements were significantly higher around flares.

Keywords. Heavy Metals, Industrial Areas, Oil Flare, Basra Province, Pollution.

1. Introduction

One of the major challenges of the past century has been the gradual accumulation of environmental pollution, and soil pollution is no exception. Soil pollution is defined as the presence, dispersion, or mixing of one or more foreign substances into the soil in such a quantity and for such a duration that it alters its physical, chemical, and biological quality in a way that is harmful to humans, other living organisms, plants, or structures and buildings.

The sources of soil pollution include agricultural, industrial, mining, petroleum, and urban activities, with heavy metals considered a part of industrial pollution. Owing to their non-degradability, bioaccumulation, ability to transfer through the food chain, and multiple sources of pollution, heavy metals pose significant environmental risks. Among these, metals such as nickel, cadmium, lead, and copper, when found in concentrations exceeding permissible limits, are considered major environmental pollutants. These metals spread into different environments,

including soil, through various human activities, such as industrial processes in the oil, gas, and petrochemical industries, and waste disposal [1].

Soil is a primary component of the environment and plays a crucial role in life and ecosystems. Heavy metal pollution in soil has severe consequences, and addressing this issue is particularly important because of the toxic properties, bioaccumulation, and long-term persistence of heavy metals in living organisms. Heavy metals were defined as elements with densities greater than 5 g cm^{-3} . These metallic elements can be categorized into two groups: essential metals (such as Cr, Co, Zn, Cu, Mn, and Fe) and non-essential metals (such as lead, cadmium, mercury, and arsenic) [2].

Heavy metals typically settle in surface soils, where they are absorbed and can contaminate of agricultural lands and natural resources, eventually entering the soil-plant-animal cycle. Consuming food products contaminated with heavy metals can increase pollutant concentrations beyond permissible thresholds, posing toxic and carcinogenic risks to plants, animals, and humans [3].

Agriculture is the first major human activity that significantly alters soil [4]. Intensive agriculture contributes to groundwater pollution, increases greenhouse gas emissions, reduces the genetic diversity of crops, and leads to eutrophication in rivers, lakes, and coastal ecosystems. Additionally, it reduces soil fertility, increases pest outbreaks in crops and disease prevalence in livestock, and increase the consumption of energy and chemical inputs [5]. For plants to grow and complete their life cycle, they need to absorb not only macronutrients, such as nitrogen, potassium, phosphorus, sulfur, calcium, and magnesium, but also essential micronutrients. In some cases, soils lack heavy metals, such as Co, Cu, Fe, Mn, Mo, Ni, and Zn, which are necessary for plant health and growth. Under such conditions, plants produced in deficient soils may negatively affect the organisms that consume them. A wide range of methods exists for identifying soil pollution. These methods typically aim to determine pollution levels by comparing different areas, applying statistical analyses, or using reference concentrations. Based on this approach, these methods have been classified into two main categories: qualitative methods, such as principal component analysis and factor analysis, and quantitative methods, such as enrichment factors and geo-accumulation index. Quantitative methods have been widely used. Another classification divides soil pollution assessment methods into individual evaluation techniques, such as the enrichment factor, pollution factor, geo-accumulation index, and risk assessment factor, which separately evaluate heavy-metal pollution. In contrast, integrated methods, such as the weighted mean pollution index and Nemero index, assess the cumulative effect of multiple heavy metals [6]. Because quantitative soil pollution assessment methods rely on reference concentration values, determining appropriate reference values for unpolluted soils is a major challenge. Common reference concentrations include background, crustal, and regulatory reference values, with the most suitable being the background concentration of the study area.

The background concentration from external regions was not ideal for evaluating areas with low pollution levels. However, a more precise and specific method involves using the background concentration within the region for soil pollution assessment. In addition to quantitative methods, approaches that consider both pollution levels and the continuous nature of variations in soil contamination can provide more accurate representations of the extent and intensity of pollution. This perspective involves the application of multivariate analyses and geostatistical methods to identify contaminated soils and define pollution classes [6]. Contamination of water and soil with heavy metals is one of the most critical challenges in the environmental sciences [7]. Heavy metals enter the environment from various sources, including the expansion of industrial

activities and the resulting increase in wastewater and waste production, extensive use of chemical and organic substances in agriculture, and combustion of fossil fuels [8].

Heavy metals pose a significant threat to plant, animal, and human health owing to their potential toxicity, high stability, and long-term effects on ecosystems. Among them, certain metals such as cadmium, lead, and chromium have no known biological role, whereas others, such as copper, zinc, and manganese are essential in small amounts for normal plant growth.

However, when present at slightly higher concentrations than required, they not only become toxic but also carcinogenic and mutagenic. Heavy metals negatively affect photosynthesis, biomass production, and plant growth [9]. Using land and water contaminated with heavy metals for agriculture not only reduces natural fertility but also leads to the contamination of food products, posing a severe risk to human health. Researchers agree that heavy metal accumulation in plants can cause various physiological, structural, and biochemical changes [10]. Pb and Cd are two prominent heavy metals that pose public health threats and are closely linked to various industrial and agricultural activities. Pb typically enters the environment through wastewater discharge into soil, the burning of fossil fuels, and its presence in fertilizers and pesticides [8]. Approximately 80% of atmospheric lead originates from the combustion of gasoline. Owing to its low solubility in water, Pb tends to accumulate in the surface soil layers. The ionic diversity of Pb is relatively simple, with Pb^{+2} being the most soluble form. From an environmental perspective, Pb is particularly significant because it mimics many aspects of calcium metabolism. High-level human exposure to this element causes severe damage to all organs and systems, including the central nervous system, blood, and kidneys, ultimately leading to premature death [11]. Cd is a highly toxic metal that is now recognized as a major environmental pollutant, posing serious toxicity risks to both animals and plants. Similar to Pb, Cd enters the environment through various industrial and agricultural activities [11]. This element is highly mobile in the soil and can be easily absorbed by plant roots and translocated to aerial organs [12]. Cadmium is a particularly concerning heavy metal because it can accumulate in plant tissues without causing visible toxicity symptoms, thereby entering the food chain unnoticed. Its toxicity in plants results from interactions with the sulfhydryl groups in enzymes and proteins. Additionally, high cadmium levels disrupt metabolic processes, increasing the production of reactive oxygen species in cells and leading to oxidative stress in plants [13].

The aim of this study was to: 1. Assess the accumulation of heavy metals in the agricultural soil of farmlands affected by oil rigs in oil wells in southern Iraq. 2. Measuring the concentrations of heavy metals, including iron, zinc, copper, manganese, lead, and nickel, in the soil of orchards surrounding oil rigs in southern Iraq.

2. Materials and Methods

2.1. Characteristics of the Study Area

2.1.1. Geography of Basra Province

Basra Province covers an area of approximately 20,720 square kilometers, with a hot and arid climate. It is located in southern Iraq and shares international borders with Iran and Kuwaiti. The capital city, Basra, is the second-largest city in Iraq after Baghdad, with a population of over two million people. The Majnoon oil field, situated in southern Iraq near the city of Basra, is major oil reserve in the country [14]. The Russian company Lukoil operates the West Qurna-2 oil field in Iraq and aims to increase production in this economically vital region.

Basra is built on an alluvial plain with sedimentary soils, and its elevation ranges from 1 to 1.08 meters above sea level. The city is surrounded by extensive palm groves, with approximately 18 million date palm trees [15].

Basra is situated in a fertile region, that has facilitated the development of its farming sector. The province produces crops, such as dates, wheat, barley, rice, sugar beets, corn, tomatoes, millet, and tobacco. Livestock, beekeeping, and fish farming are prominent economic activities. Historically, Basra was the world's primary center for date production.

In the past 12 years, oil extraction has commenced in this region, with drilling rigs burning a portion of the condensates and gases emitted from oil wells, releasing the remaining pollutants into the air, and consequently, the surrounding environment. This study examines the impact of oil-related emissions on the soils of the region.

2.1.2. Sampling

In this study, the locations of the sampling points were determined and entered into the GPS device. Based on the designed network, taking into account the ease of access and navigation, the sampling points recorded in the GPS device and the exact locations of the samples were determined, and soil sampling was carried out in the orchards around the Luke Company in the west of the village of Al-Qarna and north of Basra, located in southern Iraq. A total of 35 soil samples were collected from the date palm orchards around the oil rigs in the envelope assigned to each point and were transferred to the laboratory for physical and chemical analyses and measurement of heavy metal concentrations.

Soil samples were digested with 1 M nitric acid, the clear solution was filtered and made up to 50 ml in volume, and the concentrations of heavy metals including iron, zinc, copper, manganese, nickel, and lead were measured using an atomic absorption spectrometer [16]. After the experiments related to the measurement of heavy elements were performed, the obtained values were saved in an Excel file.

To perform statistical analyses among the sampling points studied, the following four treatments were examined:

2.2. Treatments Under Study

First treatment: Samples from stations 15, 16, 17, and 18 around rig number 1, the distances from these points to rig number 1 were 18, 18, 24 and 24 km, respectively.

Second treatment: Samples from stations 33, 34, and 35 around rig number 2, the distances from these points to rig number 2 were 10, 12, and 14 km, respectively.

Third treatment: Samples from stations 7 and 8 around rig number 3, the distance from these points to rig number 3 was 12 and 13 km, respectively.

Fourth treatment: Samples from stations 21, 22 and 23 which were far from the oil rigs and were considered control treatments. The average distance from these points to the oil rigs was 15 - 20 km. Statistical analyses were performed using SPSS version 26 software, and graphs were drawn using Excel and Origin Pro software.

3. Results

Table (1) presents some physical and chemical properties of soil at several stations in the study area, along with descriptive statistics of heavy metal concentrations in soil samples from gardens around oil drilling rigs as shown in Table (2) The elements investigated including iron, zinc, copper, manganese, nickel, and lead.

These statistics included the mean, minimum, maximum, standard deviation, mean squares, total squares, and coefficient of variation.

Finally, the spatial distribution pattern of heavy metals in the soil was mapped using the GIS software. The results are illustrated in figure 1 - 6.

To assess the risk and raise awareness of the levels of heavy metals present in soil samples from gardens around oil drilling rigs, laboratory, statistical, and descriptive examinations were conducted, the results of which are presented in the form of tables, charts, and images, and will be discussed further.

Table (1) includes some physical and chemical properties of the soil in the study area; these results were randomly obtained from soil samples taken from five stations in the cultivated area.

As shown in Table (1) the soils in the study area were calcareous and some were very calcareous, with an average lime content of 33.5% measured at the study stations. They had an alkaline pH; in terms of salinity, they fell into the very saline category; the average soluble sodium was 916.9 ppm. The soils in the study area were mainly clay loam (Table 1).

Table 1. Physical and chemical properties of soil in some stations of the study area.

Texture	%		%	Na(mg/kg)	mg/kg		TN (%)	EC (dS m ⁻¹)	pH	Station	
	C	Si	S		CaCO ₃	K					P
L	35.27	82.40	84.31	95.42	1661.0	218.1	18.0	11.0	6.23	5.7	1
C.L	47.33	9.44	63.21	95.43	719.7	132.0	15.0	24.0	5.26	4.7	7
C.L	43.31	65.32	92.35	7.20	879.6	86.2	32.0	20.0	7.36	4.7	17
S.L	14.17	57.28	29.54	6.34	356.6	38.4	4.0	15.0	3/10	4.7	27
C.L	55.37	78.38	67.23	2.26	967.6	72.6	6.0	36.0	8.48	6.9	34
C.L	39.29	14.37	47.33	5.33	916.9	109.5	15.0	21.0	2.29	3.7	Mean

Descriptive statistics obtained from measuring the concentration of heavy metals in the soil of orchards around oil rigs are shown in table (2). Table (3) presents the standard values of heavy metal concentrations in agricultural soils from several countries for comparison with the results of this research.

Table 2. Descriptive statistics of heavy metal concentration measurements studied in the soil of orchards around oil rig towers in the area.

Variable	Milligrams per kilogram (ppm)					
	Ni	Mn	Cu	Zn	Fe	Pb
Sample Size	12	12	12	12	12	12
Mean	9.96	1.673	9.19	6.102	1370	1.45
Maximum	1.114	5.885	3.24	1.177	1863	0.63
Minimum	4.58	4.450	3.13	72.70	5.491	2.25
Standard Deviation	5.32	3.240	0.4	2.30	7.474	8.11
Coefficient of Variation	5.33	7.235	2.20	49.29	6.34	2.26

Appendix (1) provides the analysis of variance of heavy metals present in the soil of orchards around oil rigs in the studied treatments.

In appendix (2), the comparison of the average concentration (mg kg⁻¹) of heavy metals in the treatments studied in the soil around oil rigs is presented, and their differences are significant at the 0.001 (**) and 0.005 (*) levels.

Table (3) the maximum allowable concentrations of the studied heavy metals in agricultural soils based on the standards of various countries.

Table 3. Maximum permissible concentration of heavy metals in agricultural soils based on standards of different countries (mg kg^{-1}).

Country/ Heavy Metal	Zn	Pb	Ni	Cu	Mn	Fe
Australia (1 & 3)	200	300	60	100	NR	NR
Canada(1 & 3)	500	200	100	150	NR	NR
China(1 & 3)	200-300	80	40-60	50-200	(4)687	NR
Germany(1 & 3)	600	1000	200	200	NR	NR
Netherlands(1 & 3)	720	530	100	190	NR	NR
Tanzania(1 & 3)	150	200	100	200	NR	NR
America(1 & 3)	1100	200	72	270	(5)525	NR
World Health Organization ¹ WHO	300	100	50	100	2000	50000
Iranian Environmental Organization(2)	500	75	110	200	NR	NR
		[17]				
		[18]				
		[19]				
		[20]				
		[21]				
		NR: Not Reported				

Table 4. Comparison of Mean Concentration (mg kg^{-1}) of Heavy Metals in Oil rigs in the Treatments Studied.

Treatment/heavy metal	Fe	Pb	Cu	Ni	Zn	Mn
1	^a 1757.1	^a 51.3	^a 21.3	^{ab} 108.2	^b 101.3	^b 630.6
2	^a 1641.8	^a 49.7	^a 23.3	^a 138.9	^a 152.1	^a 804.82
3	^b 1417.6	^a 51.7	^a 22.0	^{bc} 88.3	^b 102.9	^a 756.7
Witness	^c 624.6	^b 27.3	^b 13.8	^c 62.64	^c 71.1	^c 490.5
Significance level	**	**	**	*	**	**

A comparison of the average data for each heavy metal in the soil of the orchards around the oil rigs (milligrams per kilogram) is presented in figures 1 to 6. As shown in figure (1) the average amount of Fe measured in the soil of the orchards around the oil rigs differed between the studied treatments and the control, and the difference was significant at the 0.001 level. However, no significant differences were observed between Treatments 1 and 2.

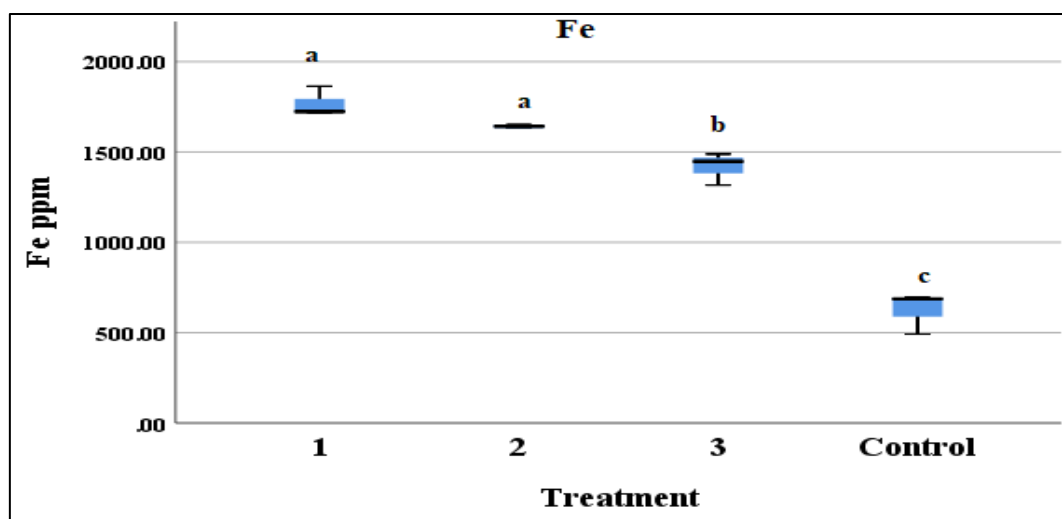


Figure 1. Comparison of the average iron content in the soil of orchards around oil rigs (mg kg⁻¹).

As shown in Figure (2) the average zinc content measured in the soil of the gardens around the oil rigs differed between the studied treatments and the control, and their difference was significant at the 0.001 level. However, no significant differences were observed between Treatments 1 and 3.

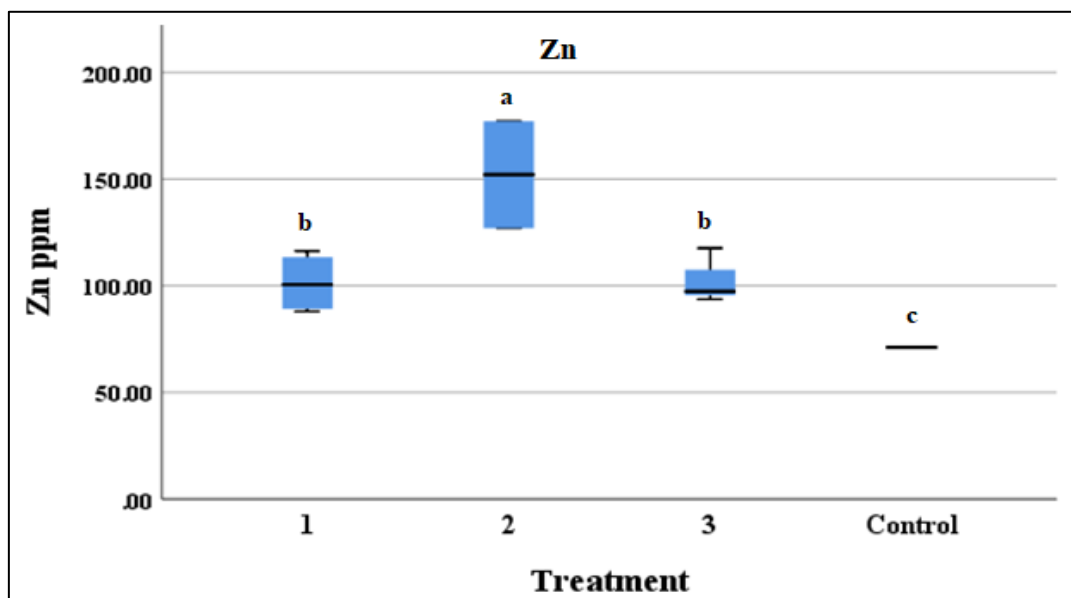


Figure 2. Comparison of average zinc levels in the soil of gardens around oil rigs (mg kg⁻¹).

As shown in Figure 3, the average amount of Co measured in the soil of the orchards around the oil rigs differed between the studied treatments and the control, and the difference was significant at the 0.001 level. However, no significant difference was observed among treatments 1, 2, and 3.

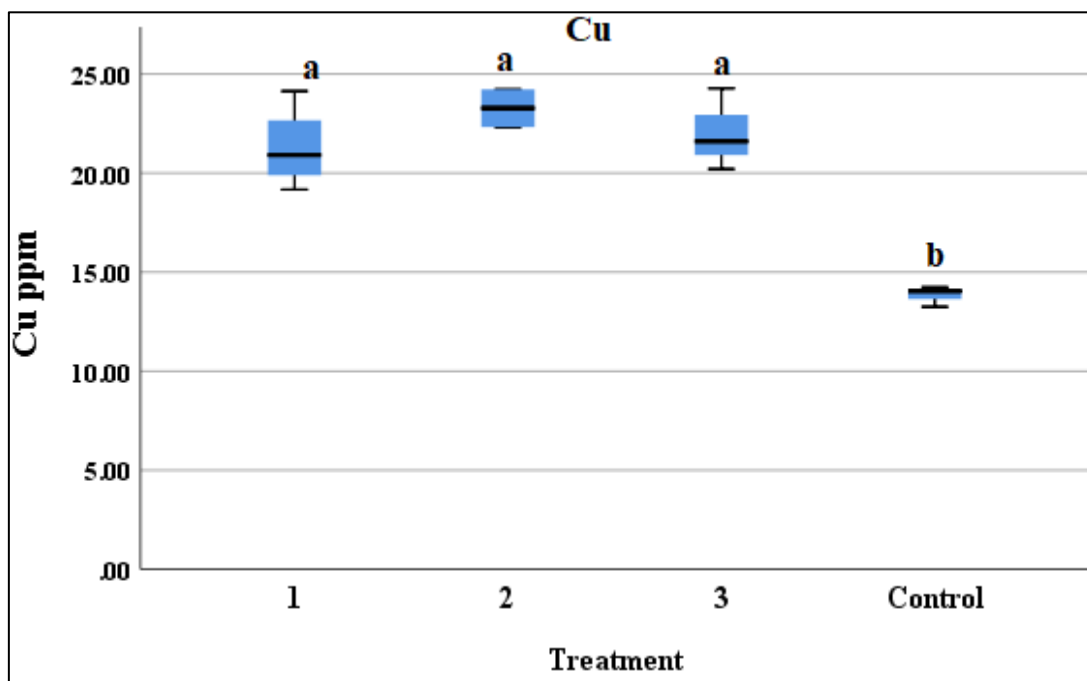


Figure 3. Comparison of the average copper content in the soil of orchards around oil rigs in the study area (mg kg⁻¹).

As shown in Figure 4, the average Mn content measured in the soil of the orchards around the oil rigs differed between the studied treatments and the control, and this difference was significant at the 0.001 level. However, no significant difference were observed between Treatments 1 and 2.

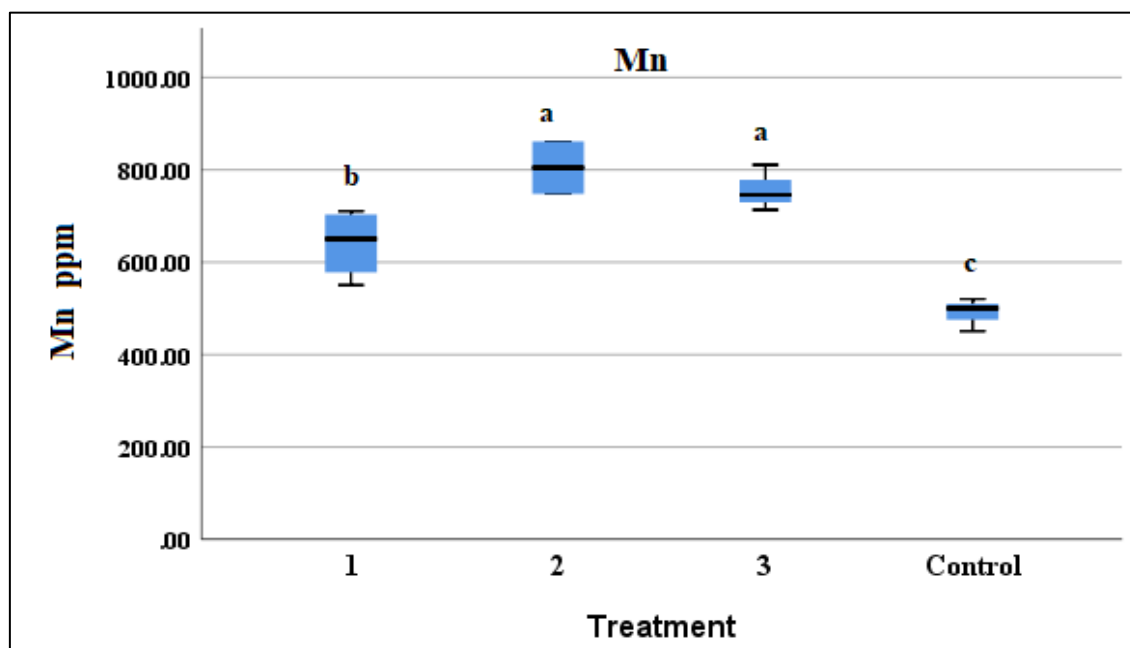


Figure 4. Comparison of the average manganese content in the soil of orchards around oil rigs in the study area (mg kg⁻¹).

As shown in Figure (5) the average measured Ni content in the soil of orchards surrounding oil rigs differed between the studied treatments and the control, with this difference being significant at the 0.001 level.

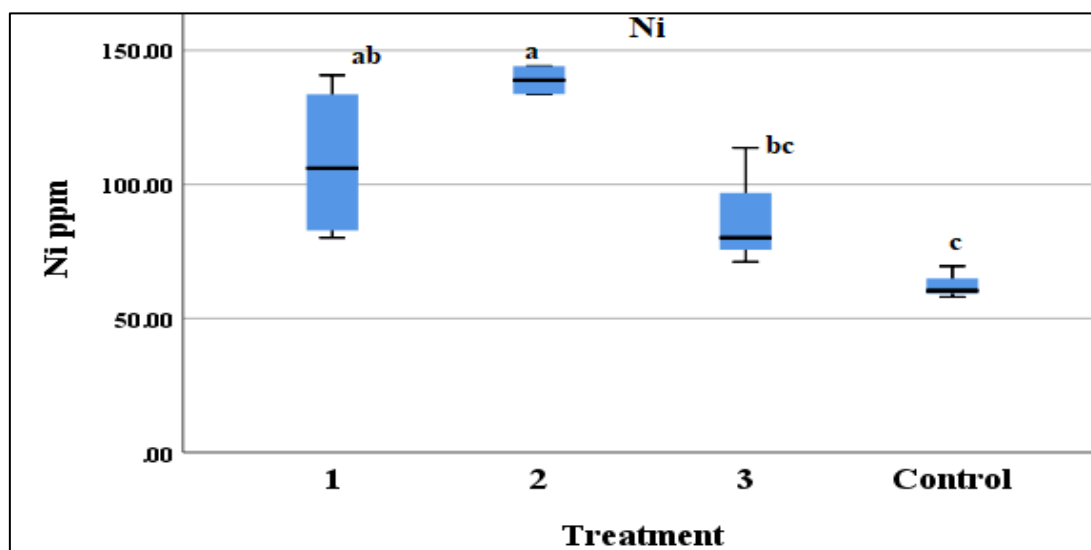


Figure 5. Comparison of the average nickel content in the soil of orchards around oil rigs in the study area (mg kg^{-1}).

As shown in Figure (6) the average measured Pb content in the soil of orchards surrounding oil rigs differed between the studied treatments and the control, with this difference being significant at the 0.001 level. However, no significant difference was observed among treatments 1, 2, and 3.

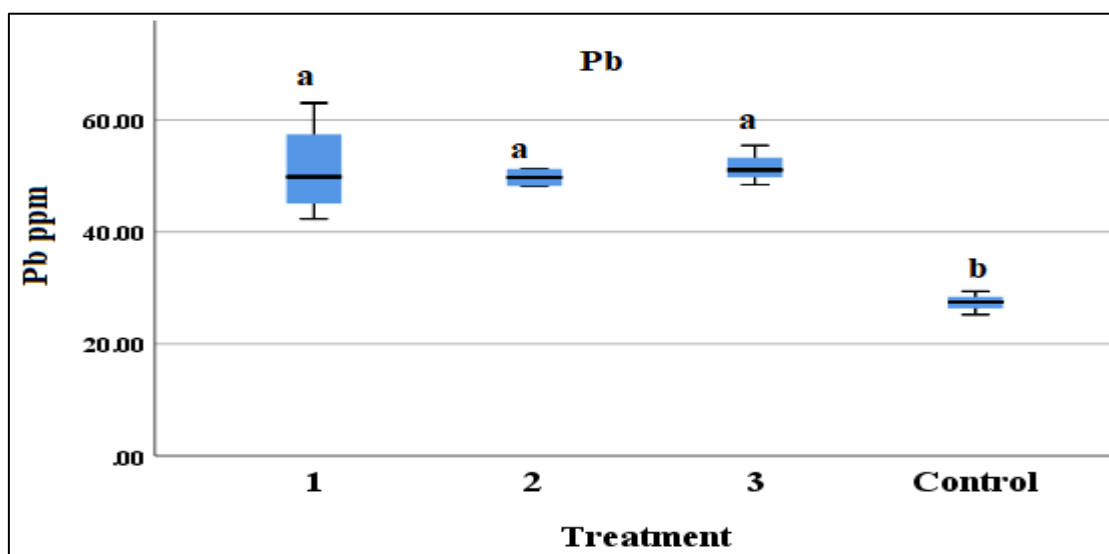


Figure 6. Comparison of the average lead content in the soil of orchards surrounding oil rigs in the studied area (mg kg^{-1}).

Appendix (2) presents the correlation coefficient between the heavy metals in the soil of the orchards surrounding the oil rigs. Accordingly, their correlation was significant at the 1% and 5% levels, respectively.

In this study, the total concentrations of heavy metals, including lead (Pb), zinc (Zn), copper (Cu), nickel (Ni), iron (Fe), and manganese (Mn) in 35 soil samples collected from the area surrounding active oil flare stacks in Basra Province, Iraq.

4. Discussion of Heavy Metal Concentrations in Orchard Soils Near Oil Flares

The average concentrations of Fe, Zn, Cu, Mn, Ni, and Pb in the soil were measured at 1370, 102.6, 19.9, 673.1, 45.1, and 96.9 mg kg⁻¹, respectively. The maximum concentrations recorded were 1863, 177.1, 24.3, 885.5, 63, and 144.1 mg/kg, whereas the minimum values were 491.5, 70.2, 13.3, 450.4, 25.2, and 58 mg kg⁻¹, respectively (Table 2). A significant positive correlation was observed between the metals (Appendix 2), indicating an interaction between different elements. Analysis of variance (ANOVA) confirmed significant differences between the different treatment groups. Three sample treatments were considered: soils surrounding oil flare stacks in the south, west, and east of the region were compared with control soils from the northern part of the area, which are far from active oil wells and are not affected by petroleum pollution.

The highest concentrations of heavy metals were found in the southern part of the region, where three active flare stacks were located. In contrast, the western and eastern sections, each with two active flare stacks, have comparatively lower concentrations. A comparison of soil heavy metal concentrations (Table 2) with the permissible limits set by the World Health Organization (WHO) and the Iranian Environmental Protection Organization (Table 3) revealed that only nickel (Ni) exceeded the WHO limit (93 mg kg⁻¹ vs. 50 mg kg⁻¹). The concentrations of other metals remained below the regulatory limits. However, at some sampling points, Ni concentrations exceeded permissible levels, and the maximum recorded concentration of Ni was 145 mg kg⁻¹. This suggests that a significant number of sampled sites had Ni concentrations above the WHO safety threshold.

Studies by [22] on agricultural soils near gas industries in Kurdistan, Iraq, found that gas plant emissions contributed to increased heavy metal concentrations. Additionally, long-term agricultural activities, including the use of fertilizers and pesticides, have led to metal accumulation and persistence in soil.

Similarly, [23] studied the spatial and temporal distribution of heavy metals in urban and suburban soil samples and, identified significant variations. Their study found that copper (Cu), lead (Pb), and zinc (Zn) were linked to industrial and traffic emissions, whereas nickel (Ni) and manganese (Mn) had natural sources.

Conclusions

In a study of the interaction of metals with one another, a positive and significant correlation was observed (Appendix 2). The analysis of variance of the heavy metal means showed that there was a significant difference between the heavy metals in the soils around the towers and the control soils that were far from the towers, with the concentration of these elements being significantly higher around the towers.

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Appendixes

Appendix 1. Analysis of variance of heavy metals present in the soil of orchards around oil rigs in the region.

Heavy Metals	Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F-Value	Significance Level
Iron	Treatment	3	2420805	806935	111.34	0.000
	Error	8	57982	7248		
	Total	11	2478786			
Zinc	Treatment	3	7883	2627.6	9.59	0.005
	Error	8	2191	273.9		
	Total	11	19974			
Copper	Treatment	3	153.85	51.28	17.16	0.001
	Error	8	23.91	2.98		
	Total	11	177.76			
Manganese	Treatment	3	157723	52574	13.52	0.002
	Error	8	31113	3889		
	Total	11	188836			
Nickel	Treatment		7784	2594.6	5.42	0.025
	Error		3830	478.8		
	Total		11614			
Lead	Treatment		1267.6	422.53	12.58	0.002
	Error		268.6	33.59		
	Total		1536.3			

Appendix 2. (6)Pearson correlation coefficient between heavy metals in garden soil around oil rigs

Metal	Manganese	Zinc	Nickel	Copper	Lead	Iron
Manganese	1					
Zinc	0.814*	1				
Nickel	0.669*	0.870**	1			
Copper	0.867**	0.733**	0.720**	1		
Lead	0.820**	0.622*	0.688**	0.921**	1	
Iron	0.623*	0.600*	0.687*	0.848**	0.822**	1

**, * Significant correlation at the 5 and 1 percent levels, respectively

Sustainable Extraction and Characterization of Cellulose Nanocrystals from Agricultural Residues (Khastawi date seeds): Physical and Chemical Insights

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Abstract. The rising demand for sustainable materials has propelled research into nanocellulose, a biodegradable nanomaterial sourced from renewables, due to its remarkable attributes, including high crystallinity, mechanical strength, and thermal stability. This study investigates the extraction of nanocellulose from Iraqi date palm (Khastawi) seed waste, a plentiful lignocellulosic biomass, to mitigate environmental issues and fulfill the demand for sustainable products. The procedure entailed a series of treatments: mechanical crushing, alkaline treatment (4% NaOH at 80°C for 2 hours), bleaching (8% NaClO for 48 hours), and acid hydrolysis (30% H₂SO₄ at 45°C for 1 hour), culminating in sonication to extract nanocrystalline cellulose (NC). The extracted NCC was characterized using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) following chemical treatment. FTIR examination verified the existence of cellulose by distinctive peaks, including O-H stretching (3469.57 cm⁻¹) and C-O vibrations (1000–1200 cm⁻¹), signifying the elimination of lignin and hemicellulose. The XRD data indicated a crystallinity index of 33.10% with prominent peaks at $2\theta = 15.98^\circ$, 20.13° , and 23.71° , validating the effective extraction of NC. This study emphasizes the feasibility of utilizing date palm seed waste as a source for nanocellulose manufacturing, providing a sustainable waste management solution and facilitating the advancement of high-value applications in packaging, composites, and textiles. The results correspond with worldwide initiatives to substitute synthetic materials with biodegradable options, highlighting the economic and environmental advantages of employing agro-waste for the synthesis of sophisticated materials.

Keywords. Nanocellulose, Date palm waste, Acid hydrolysis, Sustainable materials, Lignocellulosic biomass.

1. Introduction

The growing emphasis on utilizing natural biopolymer fibers instead of synthetic fibers has encouraged researchers to explore different sources of biodegradable natural fibers. Among the various reachable options, cellulose is extremely marked out as an essential renewable

biopolymer fiber. Cellulose consists mainly of d-glucose and has a linear and unbranched homopolysaccharide structure. It is crucial to notice that biopolymer demonstrates notable thermal and mechanical stabilities while exhibiting minimal environmental toxicity [1].

In contrast to cellulose, nanocellulose has different advantages, such as a high degree of crystallinity, large surface area, ease of surface modification, enhanced mechanical properties, improved thermal stability, and better barrier properties [2]. The diverse features of nanocellulose have paved the way a variety of applications, such as sustainable packaging materials, environmentally friendly coatings, aerogels, polymer nanocomposites, advanced electronic components, insulation materials, and high-performance textiles [3].

Nano-Cellulose (NC) is widely used as a reinforcing agent in polymer composites owing to its remarkable mechanical and thermal properties. The physicochemical, thermal, and insulating properties of different biodegradable polymers are greatly improved by the addition of NC, making them suitable for a variety of applications. [4]. To produce cellulose nanocrystals (NCs), various types of lignocellulosic waste materials have been used under certain conditions, as implemented in several previous studies [5].

To produce cellulose nanocrystals (NCs), various types of lignocellulosic waste materials have been used under certain conditions implemented in several previous studies [2,6,7]. Both crystalline and amorphous areas were present in cellulose. Crystalline particles, known as cellulose nanocrystals (NCs), are created when the amorphous portion is removed by acid hydrolysis [8].

Globally, Iraq is characterized by being one of the most important sources of palm dates, and has historically held historical importance as a domestication site for this crop. Undoubtedly, Iraq is the origin of the date palm, supported by historical records and genetic testing. It is also known as *Phoenix dactylifera* [9]. This tree has been domesticated and cultivated in Iraq since 4,000 B.C... Moreover, this country has a valued background history, and the consumption of palm trees has played a vital role in its culture. These dates, which make up a significant agricultural crop and are featured on the Iraqi banknotes, represent the nation's identity. This tree was extensively used by the people of Iraq for furniture making, medicine, and various household tools [10]. Following planting or fruit harvesting, trees generate over 200,000 tons of lignocellulosic waste annually (branches, stalks, seeds, and trunks). These agricultural wastes are used to make inexpensive, eco-friendly materials for a variety of uses, such as luggage, sports equipment, clothing, and vehicle parts [11–13].

Consequently, seasonal trimming and refinement of palms generated a significant amount of waste biomass. An estimated 20–35 kg of biomass is produced per palm tree, resulting in approximately one million metric tons of biomass waste each year (14, 15). Lignocellulosic waste biomass is a viable source of cellulosic materials for various uses. Additionally, this can address the problem of solid waste disposal. In addition, they generate additional income sources for farmers. Recent research endeavours have been developed to extract pure cellulose from particular date palm sections, such as alkali treatment, sodium hypochlorite bleaching, and acid hydrolysis, which have been used to extract microcellulose from date seeds. Type I cellulose was produced with a crystallinity index of 62%, which then increased to 72% following acid hydrolysis [16]. To date, there is a lack of studies on the isolation of NCs from date palm seeds. Because of the paucity of data regarding the extraction of NC from palm tree seed waste, the purpose of this study was to create NC using fibers from the leftover seeds of the Iraqi date palm *P. dactylifera*, which can pose health and environmental risks [1].

2. Materials and Methods

The Khastawi date seeds of “the Iraqi date palm *P. dactylifera* L.” were collected from Anbar’s orchard. Sodium hypochlorite with purity (80%) (one type of Sigma-Aldrich product) and the percentage was 8% and already taken (250 ml), sodium hydroxide with purity (97%) (Sigma-Aldrich), and sulfuric acid with purity (95%–97%) (one type of Sigma-Aldrich product), and a percentage of 30% was used as purchased from the supplier.

2.1. The Processing of Nanocellulose Extraction

Some processing steps have been performed to obtain nanocellulose, such as cleaning and some treatments, as shown in figure (1)

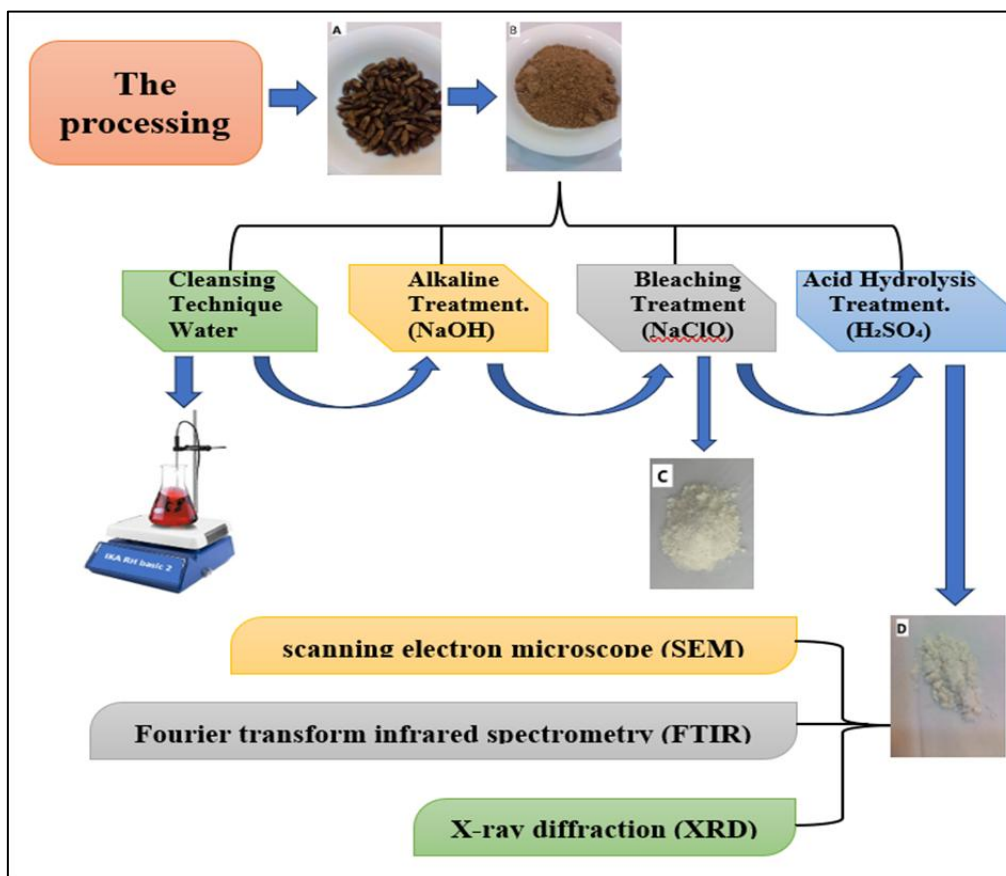


Figure 1. The diagram of nanocellulose (NC) processing.

2.2. Cleansing Technique

As illustrated in figure (2), the date palm seeds were mechanically crushed in a standard blender and then immersed in water at 100°C while being agitated using a magnetic stirrer device (IKA RH basic 2 model) set to 300 rpm for an hour to remove any remaining solutes. It was subsequently ground into a fine powder after vacuum drying for 15 h at 50°C.



Figure 2. Magnetic stirrer device (IKA RH basic 2 model).

2.3. Alkaline Treatment

The fine seed powder was exposed to a 4 wt.% sodium hydroxide (NaOH) solution for two hours at 80°C to extract pure cellulose. After several rounds of washing with distilled water until the pH reached 7, the resulting liquid was filtered and dried.

2.4. Bleaching Treatment

After bleaching the samples with 8% sodium hypochlorite (NaClO) for 48 h at room temperature, they were filtered and repeatedly cleaned with distilled water until the pH reached 7. The purpose of these procedures is to degrade the dye molecules and phenolic chemicals found in lignin. After drying, the samples were ground into a fine powder.

2.5. Treatment by Acid Hydrolysis

Acid hydrolysis is the final stage of the extraction of nanocrystalline cellulose. For one hour at 45°C with constant stirring, the finely ground seed powder was treated with 30 wt percent sulfuric acid (H₂SO₄) solution. To ensure that the acid was removed, the resultant mixture was filtered and thoroughly cleaned with distilled water before dehydration in a vacuum oven for 24 h at 50°C. [17].

The suspension was sonicated with an Ultra Sonicator (UP400S) for 1 h and stored for further processing, as shown in figure (3 A-D)



Figure 3. A- Dates before grinding and crushing, B- Dates after grinding and crushing, C- Date powder after shortening, D- Nanocellulose powder.

3. Results and Discussion

After made all processing on the powder of cellulose, the nanomaterials tests had done like “scanning electron microscope (SEM)” test, “fourier transform infrared spectrometry (FTIR)” test and “X-ray diffraction (XRD)” analysis. Thus, the results showed that the powder obtained was a nanomaterial as the follow:

3.1. SEM Analysis

Table 1. The conditions of SEM analysis.

Figures	EHD flow	Particles size	Working distance (wd)	Magnification
Figure 1-a	10 kv	200 nm	5.1 mm	20 kx
Figure 1-b	10 kv	200 nm	5.1 mm	50 kx
Figure 1-c	10 kv	200 nm	5.1 mm	20 kx
Figure 1-d	10 kv	1 μ m	5.1 mm	10 kx
Figure 1-e	10 kv	10 μ m	5.1 mm	1 kx
Figure 1-f	10 kv	1 μ m	5.1 mm	10 kx

SEM (Hitachi) fitted with energy dispersive X-ray (EDX) was used to study the morphology of the chemically treated biomass; EDX was utilized to analyze the elements that identified samples at various magnifications, as shown in Table (1) for condition (1) and figure (4 a-f).

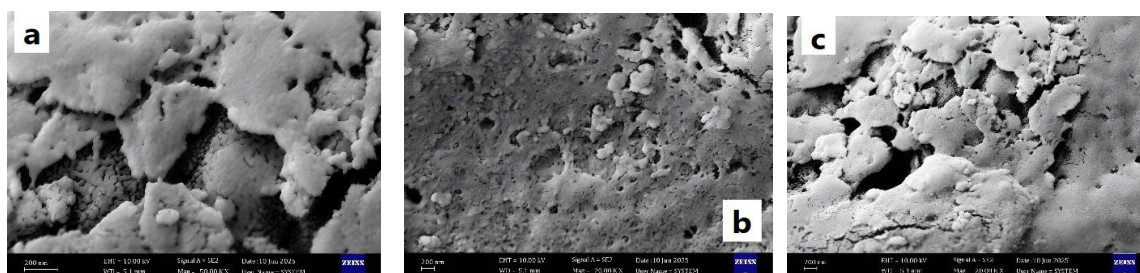


Figure 4(a-c). SEM test of nanocellulose with EHD=10 kv, particles size= 200 nm and magnification around (10-50) kx.

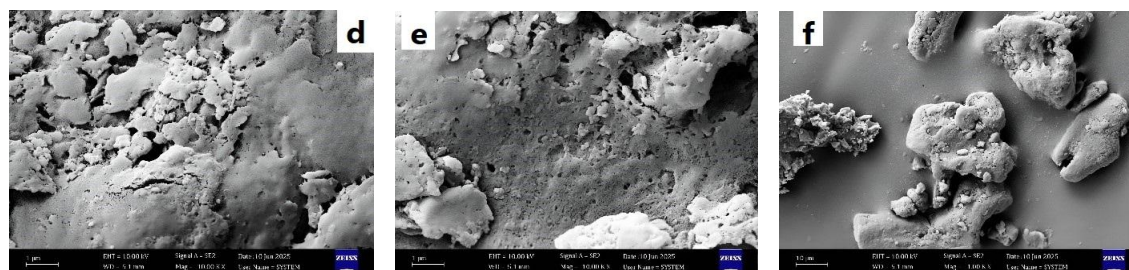


Figure 4(d-f). SEM test of nanocellulose with EHD=10 kv, particles size= (1-10) μ m and magnification.

A thin film of gold was sprayed over a dry powder sample that had been placed on carbon tape in an argon environment with an accelerating voltage of 6 kV. Elemental analysis by EDX was performed at an accelerating voltage of 10 kV, as shown in figure (5 a-b). An EHD-induced circulation flow (EHD flow) was applied at a voltage of 10 kV for all samples. The particles had sizes ranging between 200 μ m and 10 μ m. The material appears to be cleaner, purer, and more biodegradable when nanocellulose is synthesized using a combination of NaOH and NaClO. This

is because cellulose may be extensively cleaned by the NaClO concentration until it is white in color and all dirt traces disappear, whereas the NaOH content can swiftly break down organic materials. The contaminants in the sample vanish [18].

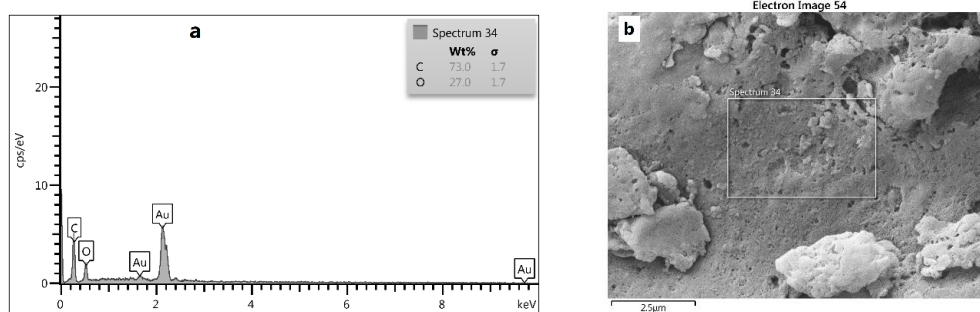


Figure 5. The elemental analysis by EDX after sputtered with a thin gold layer under an argon atmosphere.

3.2. Fourier Transform Infrared Spectrometry (FTIR)

Based on their distinct spectral signatures, FTIR can be used to distinguish between various forms of nanocellulose, such as cellulose nanocrystals and cellulose nanofibrils. Additionally, it may be used to study how nanocellulose interacts with other elements in composite materials, giving researchers important knowledge about the chemical makeup, structure, and characteristics of these materials, which are crucial for a variety of applications.

In this study, the FTIR spectra in the transmission mode, between 4500 and 400 cm^{-1} , were recorded using a Nicolet 6700 Fourier transform infrared spectrometer. As seen in figure (6), scans of the raw material, extracted cellulose, and produced nanocellulose were all performed at a resolution of 4 cm^{-1} .

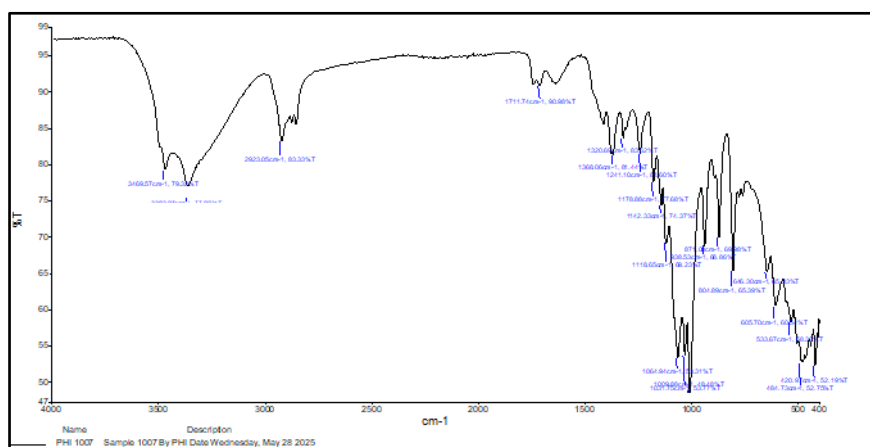


Figure 6. Emanation of the nanocellulose by using Fourier transform infrared spectrometry (FTIR).

O-H stretching vibrations are responsible for the broad band seen in the peaks at 3469.57 and 3363.02 cm^{-1} , which suggests the presence of hydrogen bonds and hydroxyl groups. The strength and type of hydrogen bonds found in the nanocellulose structure are revealed by these O-H stretching areas, which are crucial for understanding its characteristics. However, the C-H stretching vibrations of the methyl and methylene groups are linked to the peak at 2923.05 cm^{-1} .

The C-H bending and scissoring vibrations were linked to the maxima at 1368.06 and 1320.69 cm^{-1} . Particularly in the glycosidic linkages, the characteristic peaks at 1178.88, 1142.33, 1118.65, 1064.94, 1031.75, and 1009.88 were linked to C-O stretching vibrations in cellulose. These peaks are essential for determining whether cellulose is present and its structure. The sharpness and intensity of the peaks in the 1000–1200 cm^{-1} area are particularly useful for determining the nanocellulose's crystallinity.

During the nanocellulose isolation process, changes in the FTIR spectra can reveal the presence of contaminants or the elimination of other substances such as lignin or hemicellulose [19,20].

3.3. X-Ray Diffraction (XRD) Analysis

The raw material, cellulose, and nanocellulose were also observed using X-ray powder diffraction (XRD), as shown in figure (7). Measurements were performed on a D8 Brucker diffractometer with Cu K radiation ($\lambda = 1.54178 \text{ \AA}$) in the range 2θ of = 10-70° with a 0.02° step size.

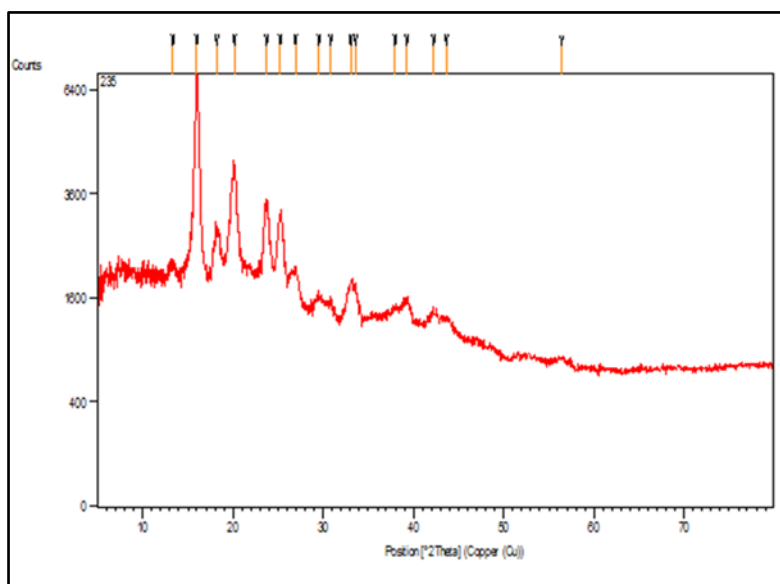


Figure 7. Emanation of the nanocellulose by X-ray diffraction (XRD).

Figure 1. had been found of the XRD examination of nanocellulose were described, which showed three peaks in the 2θ diffractogram for 4% NaOH after two hours of heating at 80°C of roughly $2\theta = 15.98, 23.71$, and 20.13 . The structural properties of cellulose include a content of approximately 66.90% amorphous and 33.10% crystalline.

Conclusion

This study successfully demonstrated the extraction of nanocellulose from Iraqi date palm seed waste using a combination of alkaline, bleaching, and acid-hydrolysis treatments. Characterization through SEM, FTIR, and XRD confirmed the formation of nanocrystalline cellulose with a crystallinity index of 33.10% and particle sizes ranging from 200 nm to 0.01 nm. The process not only provides a sustainable method for valorizing agricultural waste, but also yields a high-value material with potential applications in biodegradable composites, packaging, and textiles. By converting underutilized biomass into nanocellulose, this study contributes to eco-friendly material development and supports waste-to-wealth initiatives.

Author Contribution

Author 1: Conceptualization, Methodology, Writing- Original draft preparation.

Author 2: Software, Data curation, Supervision

Author 3: Investigation, Software, Validation, Writing- Reviewing and Editing.

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Impact of Bio- and Mineral-Fertilizers on the Levels of Available and Total Potassium in Maize (*Zea Mays* L.) Soil Cultivated

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Abstract. This study sought to examine the impact of biofertilizers and mineral fertilizers on the levels of available plants available and total potassium in soil. Yellow maize was cultivated in a silty clay soil. The experimental treatments included a control treatment, urea at 250 kg N ha⁻¹, ammonium sulfate at 200 kg N ha⁻¹, BioHealth at 4-5 kg N ha⁻¹, liquid (EM) at 400 L ha⁻¹, and a fertilizer combination consisting of one-quarter of the recommended dosage of each fertilizer. The levels of available and total potassium were assessed 70 and 100 d post-planting, both within and beyond the rhizosphere, to evaluate the impact of biofertilizers and mineral fertilizers on soil their soil availability. The findings indicated that the amalgamation of biofertilizers and mineral fertilizers yielded the best levels of available and total potassium at 70- and 100-days post-planting. In the single fertilization treatment, BioHealth surpassed the other treatments in both available and total potassium concentrations at 70 and 100 days post-planting. The concentration of available potassium in the rhizosphere soil was lower than that in the bulk soil across all treatments and at both the 70- and 100-day intervals. The findings indicated a sustained rise increase in total potassium concentration in the bulk soil across all treatments during both the 70- and 100-day measurement periods, with the greatest significant increase observed at the 100-day interval.

Keywords. Available potassium, Total potassium, Rhizosphere, Biofertilizer, Mineral fertilizer.

1. Introduction

Described the rhizosphere as the area around the root hairs or soil particles populated by several microorganisms that either coexist or compete for nutrition. Bacteria represent the predominant category of microorganisms in the root zone. These bacteria can exert either beneficial or detrimental effects influences on the growth of diverse plants in the soil, forming a region around their roots. referred to as the rhizosphere [1]. The rhizosphere exhibits significant biological activity and abundant organic matter and carbon dioxide; however, it possesses low soil reactivity, mineral content, and moisture levels. This zone, characterized by heightened microbial biological activity, represents a distinct ecosystem adept at decomposing organic matter and accumulating its byproducts. Plants derive advantages from the nutrients and water in this zone, resulting in the buildup of minerals and organic compounds owing to their activity [2].

Potassium is a vital nutrient for plant development and significantly influences production, since plants require it in substantial amounts [3]. Potassium plays a crucial role in carbon dioxide metabolism, facilitates energy synthesis via ATP. It transforms light energy into chemical energy in the form of ATP and NADPH, which are crucial for all critical functions including the synthesis of proteins, carbohydrates, and lipids. [4] demonstrated that increasing potassium intake enhances nitrogen absorption and its conversion into proteins, as potassium is crucial for protein synthesis. Moreover, potassium shortage results in the reduced absorption of other nutrients. Potassium is ranked third in significance after nitrogen and phosphorus because of its critical functions in plant nutrition, carbon metabolism, cell division, lodging resistance, and nutrient translocation from the source to the sink. is ranked third in significance after nitrogen and phosphorus.

The majority of Most crops require substantial nutrients and derive significant benefits from the use of mineral fertilizers, resulting in enhanced yields and superior quality [5,6]. Excessive use of fertilizers beyond prescribed limits may result in crop damage [7]. Alam et al. [8] showed that the incorporation of ammonium sulfate into soil decomposes it into NH_4^+ and SO_4^{2-} , which are readily available for absorption by crops. Consequently, ammonium sulfate is extensively utilized in soil.. (EM) comprises 80 types of beneficial microorganisms selected from a pool of 2,000 helpful and detrimental microorganisms [9]. Maize is utilized in various sectors including biofuel production, electricity generation, and animal feed manufacturing. It possesses significant nutritional significance for humans, as it yields oil and starch and is rich in protein, as well as vitamins A, B1, and B2 [10].

This study aimed to investigate the impact of different types of biofertilizers and mineral fertilizers on the available and total potassium concentrations in the rhizosphere of maize plants.

2. Materials and Methods

The experiment was conducted in a field located in the Al-Nuriyah sub-district of Al-Shafi'iyah district, Diwaniyah Governorate, during the fall of 2024. The soil in the field had a heterogeneous silty clay composition. A soil sample was collected prior to planting, air-dried, pulverized with a plastic hammer, and sieved through a 2 mm mesh for chemical and physical soil analysis in laboratories at the University of Kirkuk Ethical approval No.03C01L001 (Table 1).

2.1. Physical Analysis

- Volumetric Analysis of Soil Separates: It estimated using the hydrometer method, as described in [11].
- Bulk Density: T was estimated using the core sample method, as described in [11].

2.2. Chemical Analysis

- Physical Reaction (pH): The soil reaction rate was estimated in a 1:1 soil: water suspension using a pH meter, as described in [12].
- Electrical Conductivity (EC): Electrical conductivity was determined in a 1:1 soil: water suspension using an EC. meter as described in [12].
- Cation Exchange Capacity (CEC): The cation exchange capacity of cations was determined using ammonium acetate and sodium acetate. as described in [11].
- Organic Matter: Organic matter was determined by wet digestion using 1N $\text{K}_2\text{Cr}_2\text{O}_7$ according to the method described by Walkly and Blak [11].

2.2.1. Soluble Cations and Anions According to the Methods Described in [12]

- Calcium (Ca^{+2}): Calcium was determined by titration with ferrous sulfate (Na-EDTA) using ammonium parbunet reagent according to the method described by Lanyon and Heald [12].
- Magnesium (Mg^{+2}): Calcium and magnesium were measured by titration with Na-EDTA using the Ericchrome Black T reagent, and calcium was subtracted from the total calcium and magnesium according to the method of Lanyon and Heald [12].
- Sodium (Na^{+1}): Sodium content was determined using a flame photometer.
- Potassium (K^{+}): Potassium was dissolved in a 1:1 soil: water extract using a flame photometer.
- Sulfate (SO_4^{-2}): Sulfate was determined by precipitation as barium sulfate.
- Carbonates and bicarbonates (HCO_3^{-1}): Bicarbonates were determined by titration with 0.01 N sulfuric acid (H_2SO_4) in the presence of methyl orange indicator.
- Chloride (Cl^{-1}): Chloride was determined by titration with 0.01 N silver nitrate (AgNO_3) in the presence of potassium chromate, according to the method described in [13].
- Carbonate minerals: This was estimated by titration with (N1) HCl and back titration with (N1) NaOH, according to the method described in [12].
- Available Nitrogen: The NH_4^+ ion concentration was estimated by extraction with a potassium chloride solution (2N) using MgO and measured using a microKjeldahl apparatus according to the method of [11] and described in [11]. The nitrate ion concentration was estimated by reduction using a Devarda alloy and then measured using a microKjeldahl apparatus.
- Available Phosphorus: Available phosphorus in the soil was extracted using 0.05 M sodium bicarbonate (NaHCO_3) at pH 8.5, and the color was developed using aluminum molybdate and ascorbic acid. Phosphorus was measured using a spectrophotometer at 882 nm, as described previously [12].
- Available Potassium: Potassium ions were estimated by extraction with a 1N ammonium acetate solution and then measured using a flame photometer, as described in [11].
- Total Potassium: Soil samples were subjected to digestion using a combination of 48% hydrofluoric acid, 97% sulfuric acid, and perchloric acid in a 30-ml platinum beaker and subsequently heated. Total potassium was subsequently quantified using flame photometer following the methodology established by [14]. Soil plowing, thinning, and leveling were performed. The study soil was partitioned into three sectors, each including six 3×3 m plots (treatments) serving as experimental units. A separation of 1 m was maintained between sectors and 1 meter between the experimental units to avert the transfer of water and fertilizers between treatments.

The experiment was conducted according to a randomized complete block design with three replicates, each containing six randomly assigned treatments. Eighteen experimental units were 18. The experiment included the following treatments.

2.3. Control Treatment

Urea fertilizer (46% N) was applied 10 d after planting at a level of 250 kg ha^{-1} .

Ammonium sulfate fertilizer (21% N, 24% S) was applied 10 d after planting at a level of 200 kg ha^{-1} .

BioHealth fertilizer ($4\text{-}5 \text{ kg ha}^{-1}$) was applied 10 days after planting. Humintech, Germany, prepared the Bio Health fertilizer. It is a mixture of fungi, bacteria, humic acid, and seaweed, in the form of water-soluble granules.

Effective Microorganisms Liquid Biofertilizer: The spraying process was carried out manually, by diluting one liter of this fertilizer in four liters of water. This process was carried out using a 16-liter hand sprayer with five sprays, each spraying (400 liters ha⁻¹).

Fertilizer Mixture (a quarter of the recommended amount of each fertilizer).

Maize (*Zea mays* L.) seeds were sown in rows on August 17, 2024. The inter-row spacing was 75 cm and the inter-hole distance was 25 cm, with four rows per plot. Three seeds were sown in each hole and thinned to one following germination. There were 12 plants per row, yielding 48 plants in the experimental unit.

The corn stem borer was controlled using a granular diazinon herbicide, with one grain placed in the center of each leaf. Weeding was manually performed five times during the growing season. The yellow corn crop was harvested upon the emergence of signs of maturity after ear drying on December 19, 2024.

Table 1. Chemical and physical traits of the study soil before planting.

Trait		Value	Unit
Reaction rate: 1:1 pH		7.65	---
Electrical conductivity: 1:1 EC		3.0	Decisiemens. m ⁻¹
Cation exchange capacity		22.15	Centimole. kg ⁻¹
Carbonates Minerals		273	g. kg ⁻¹
Organic matter		4.68	g. kg ⁻¹
Positive dissolved ions	Ca ²⁺	13.51	mmol/L ⁻¹
	Mg ²⁺	11.00	
	Na ¹⁺	2.91	
	K ⁺	1.01	
	SO ₄ ²⁻	13.85	
Negative dissolved ions	HCO ₃ ¹⁻	1.15	mmol/L ⁻¹
	CO ₃ ²⁻	Nil	
	Cl ⁻	15	
Available Nitrogen		12	mg. kg ⁻¹
Available Phosphorus		3.18	
Available Potassium		105.321	
Bulk Density		1.25	Mica. Gram m ⁻³
Tissue Class		silty clay loam	
Volumetric analysis of soil fractions	Sand	220	g. kg ⁻¹
	Silt	368	
	Clay	415	

2.4. Statistical Analysis

The SAS Statistical Analysis System was used to analyze the data using a randomized complete block design (RCBD). Differences between means were compared using the least significant difference (LSD) test at a significance level of 0.05.

3. Results and Discussion

3.1. Available Potassium Content in the Soil 70 Days Post-Planting

Table 2 illustrates the impact of incorporating biofertilizer, mineral fertilizer, and a combination of both on the available potassium content in the rhizosphere and bulk soil 70 d post-planting. Statistical analysis results indicated significant differences at a p-value of 0.05. The combined

application of mineral fertilizers and biofertilizers surpassed all other treatments in both rhizosphere and extra-rhizosphere soils, yielding 218.32 and 237.16 mg kg⁻¹, respectively. The ammonium sulfate treatment resulted in the lowest amounts in both the rhizosphere and extra-rhizosphere soils, measuring 124.78 and 141.90 mg kg⁻¹, respectively.

Table 2. Impact of fertilizer type on available potassium concentration (mg kg⁻¹) in the rhizosphere and bulk soils 70 days post-planting.

Treatments	Soil Zone	
	Rhizosphere soil	Bulk soil
Control Treatment	131.80	154.71
Urea	127.29	148.15
Ammonium Sulfate	124.78	141.90
BioHealth	179.55	197.34
Effective Microorganisms	145.09	161.89
Bio-Mineral Fertilizer Combination	218.32	237.16
LSD 0.05	11.690	17.971

Biofertilizers (BioHealth and liquid EM biofertilizer) surpassed mineral fertilizers (urea and ammonium sulfate) in terms of potassium concentration availability. The maximum potassium content was attained with the BioHealth treatment, measuring 179.55 and 197.34 mg kg⁻¹ in the rhizosphere and bulk soils, respectively. This may be attributed to the composition of BioHealth fertilizer composition, which includes 11% water-soluble potassium (K₂O), as stated by the manufacturer, Humintech, Germany (Table 2).

Potassium concentrations were elevated with the liquid EM treatment, measuring 145.09 mg kg⁻¹ in the rhizosphere and 161.89 mg kg⁻¹ in the bulk soils. This aligns with [14], who indicated that EM biofertilizer treatment surpassed EM treatment, achieving the highest average of 164.15 mg kg⁻¹.

The statistical analysis findings indicated that the control treatment yielded greater amounts of plant-available potassium (131.80, 154.71 mg kg⁻¹) than the urea treatment (127.29, 148.15 mg kg⁻¹) and the ammonium sulfate treatment (124.78, 141.90 mg kg⁻¹). The degradation of urea results in a reduction in soil pH, which in turn leads to the fixation of potassium in an unavailable form [15].

3.2. Available Potassium Content in the Soil 100 Days Post-Planting

Table 3 illustrates the impact of incorporating biofertilizer, mineral fertilizer, and the combination of mineral fertilizers with biofertilizers on the concentration of available potassium in the rhizosphere and surrounding soil 100 days after planting. The statistical analysis results indicated significant differences at a significance level of 0.05. The combination of mineral fertilizers and biofertilizers surpassed that of all the other treatments. Ammonium sulfate treatment resulted in the lowest potassium concentrations, measuring 116.46 mg kg⁻¹ in the rhizosphere and 150.25 mg kg⁻¹ in the bulk soil. The breakdown of ammonium sulfate in the soil leads to a decrease in soil pH. This reduction results in K being sequestered inside clay minerals or in non-adsorbable exchange sites, hence thereby diminishing the concentration of available K [16]. The BioHealth treatment resulted in an elevated concentration of available potassium following the combined application of biofertilizers and mineral fertilizers. The potassium concentrations were 173.86 mg kg⁻¹ in the rhizosphere and 215.98 mg kg⁻¹ in the extra-rhizosphere.

Table 3. Impact of fertilizer type on the concentration of available potassium (mg kg^{-1}) in the rhizosphere soil and surrounding area after 100 days of planting.

Treatments	Soil Zone	
	Rhizosphere soil	Bulk soil
Control Treatment	126.83	162.09
Urea	121.16	155.53
Ammonium Sulfate	116.46	150.25
BioHealth	173.86	215.98
Effective Microorganisms	139.72	175.18
Bio-Mineral Fertilizer Combination	212.72	262.04
LSD 0.05	12.760	15.455

Figure 1 shows that the concentration of available potassium in the rhizosphere soil was lower than that outside the rhizosphere throughout all treatments and at intervals of 70 and 100 d. This results from root potassium uptake and microbial activity in the rhizosphere, thereby diminishing the potassium content in this area. This aligns with the findings of [15,18]. The findings indicated that the treatment integrating mineral fertilizers with biofertilizers yielded the maximum concentration of available potassium at intervals of 70 and 100 d. Tables (2 and 3) and Figure (1) indicate that the potassium content consistently diminished at measurement intervals of (70 and 100) in the rhizosphere soil across all treatments. This signifies a substantial rate of potassium uptake by plants during their development phases [19].

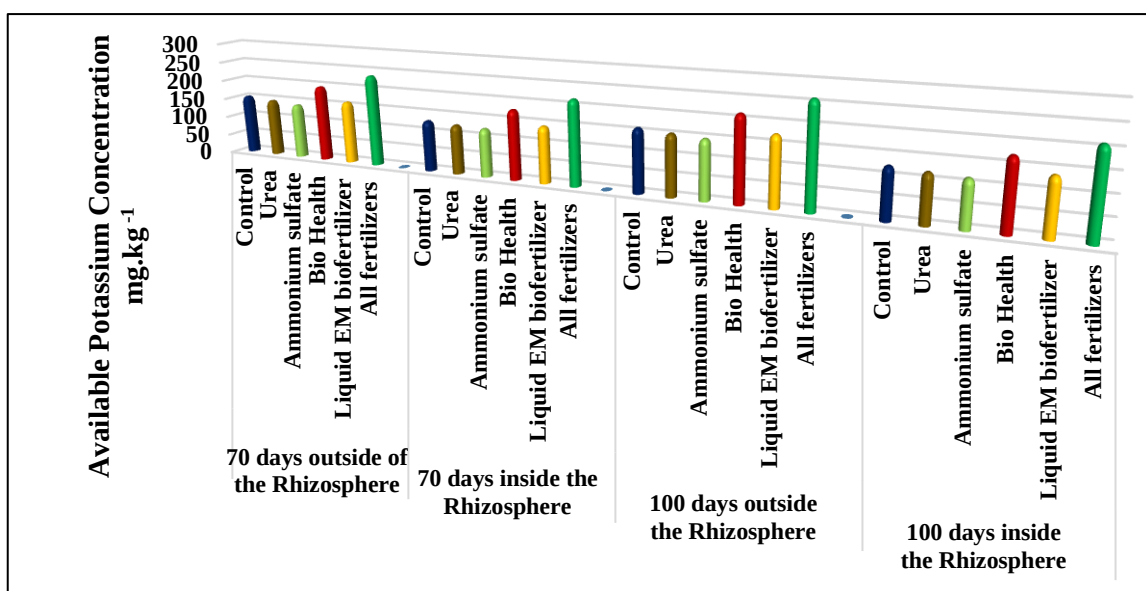


Figure 1. Influence of fertilizer type on the potassium concentration in the rhizosphere and bulk soils at 70- and 100-days post-planting (mg kg^{-1}).

The data indicate that urea treatment resulted in reduced available potassium concentrations, measuring $121.16 \text{ mg kg}^{-1}$ in the rhizosphere and $155.53 \text{ mg kg}^{-1}$ in the extra-rhizosphere soils, compared to other treatments. This contradicts the findings of [20], which indicated that urea application resulted in a substantial 51.2% increase in available Cu content in the rhizosphere soil relative to the control treatment. These findings indicate that biofertilizers enhance potassium concentrations, especially in the bulk soil. This signifies their function in enhancing nutrient

availability in a balanced manner and over extended periods, without precipitating rapid nutrient depletion, as can occur with mineral fertilizers. Conversely, treatments employing a combination of all fertilizers the best results, underscoring the significance of biodiversity in regulating plant nutrition. This outcome suggests that implementing balanced nutritional practices with biofertilizers helps preserve K levels, thereby enhancing the long-term sustainability of soil characteristics.

3.3. Total Potassium Content in the Soil 70 Days Post-Planting

Table 4 illustrates the impact of incorporating biofertilizer, mineral fertilizer, and a combination of both on the total potassium content in the rhizosphere and bulk soil 70 d post-planting. The statistical analysis results indicated significant differences at a significance level of 0.05. All interventions surpassed control therapy. The combination of all mineral fertilizers and biofertilizers resulted in the maximum total potassium content in both the rhizosphere and bulk soil. The potassium concentration was 12,890.32 mg kg⁻¹ in the rhizosphere soil and 12,935.65 mg kg⁻¹ in the bulk soil. In contrast, the control treatment recorded the lowest values of 11,408.04 mg kg⁻¹ and 11,452.85 mg kg⁻¹ in the rhizosphere and bulk soil, respectively. The ammonium sulfate treatment surpassed the urea treatment in total potassium content, achieving 11,976.23 mg kg⁻¹ and 12,034.65 mg kg⁻¹ in the rhizosphere and bulk soil, respectively. Upon treatment with urea, the total potassium concentrations were 11708.48 mg kg⁻¹ in the rhizosphere soil and 11753.12 mg kg⁻¹ in the bulk soil. The inclusion of sulfur in ammonium sulfate lowers the soil pH, enhances the solubility of potassium compounds, and facilitates their release from non-exchangeable sites on colloidal surfaces [21].

Table 4. Impact of fertilizer type on total potassium content (mg kg⁻¹) in the rhizosphere and bulk soils 70 days post-planting.

Treatments	Soil Zone	
	Rhizosphere soil	Bulk soil
Control Treatment	11408.04	11452.85
Urea	11708.48	11753.12
Ammonium Sulfate	11976.23	12034.65
BioHealth	12347.78	12413.34
Effective Microorganisms	12208.86	12268.24
Bio-Mineral Fertilizer Combination	12890.32	12935.65
LSD 0.05	166.435	169.089

The findings indicated that biofertilizers (BioHealth and Effective Microorganisms) outperformed mineral fertilizers (urea and ammonium sulfate) in terms of total potassium content. BioHealth generated 12347.8 mg kg⁻¹ in the rhizosphere and 12413.34 mg kg⁻¹ in the bulk soils. The application of effective microorganisms (EM) resulted in total potassium concentrations of 12,208.8 mg kg⁻¹ in the rhizosphere and 12,268.24 mg kg⁻¹ in the bulk soil. This is because of the successful participation of beneficial microorganisms in biofertilizers, especially those that can solubilize potassium from insoluble soil compounds, such as mica and feldspar. These organisms excrete organic acids or chelating agents that facilitate the dissolution of K-containing substances [22,23].

3.4. Total Potassium Content in the Soil 100 Days Post-Planting

Table 5 illustrates the effect of incorporating biofertilizer, mineral fertilizer, and a combination of both on the total potassium content in the rhizosphere and bulk soils after 100 days of culture.

The statistical analysis results indicated significant differences at a significance level of 0.05. All therapies surpassed the control therapy, aligning with [24]. The maximum total potassium content in the rhizosphere and bulk soils was achieved by integrating all mineral and biofertilizers. The total potassium concentrations were 12,787.65 mg kg⁻¹ and 13,089.36 mg kg⁻¹ for the rhizosphere and bulk soils, respectively, compared to the control treatment, which recorded the lowest concentrations of 11,259.73 mg kg⁻¹ and 11,566.31 mg kg⁻¹ for the rhizosphere and bulk soils, respectively. Biofertilizers surpassed mineral fertilizers in terms of total potassium content in both rhizosphere and bulk soils. This aligns with the findings of [25,26], who demonstrated that the organic fertilizer treatment produced the most significant levels of available iron and manganese, followed by the full mineral fertilizer treatment.

Table 5. Impact of fertilizer type on total potassium concentration (mg kg⁻¹) in the rhizosphere and bulk soils 100 days post-planting.

Treatments	Soil Zone	
	Rhizosphere soil	Bulk soil
Control Treatment	11259.73	11566.31
Urea	11605.45	11887.14
Ammonium Sulfate	11836.93	12151.80
BioHealth	12222.17	12536.71
Effective Microorganisms	12100.66	12399.24
Bio-Mineral Fertilizer Combination	12787.65	13089.36
LSD 0.05	167.691	168.785

The results in Tables 4 and 5 indicate that the potassium concentration consistently increased across the measurement periods of 70 and 100 days in the bulk soil across all treatments. This increase was most evident after 100 days. This may have resulted from the ongoing dissolution and degradation of potassium-rich minerals in the soil through chemical and biological mechanisms [27].

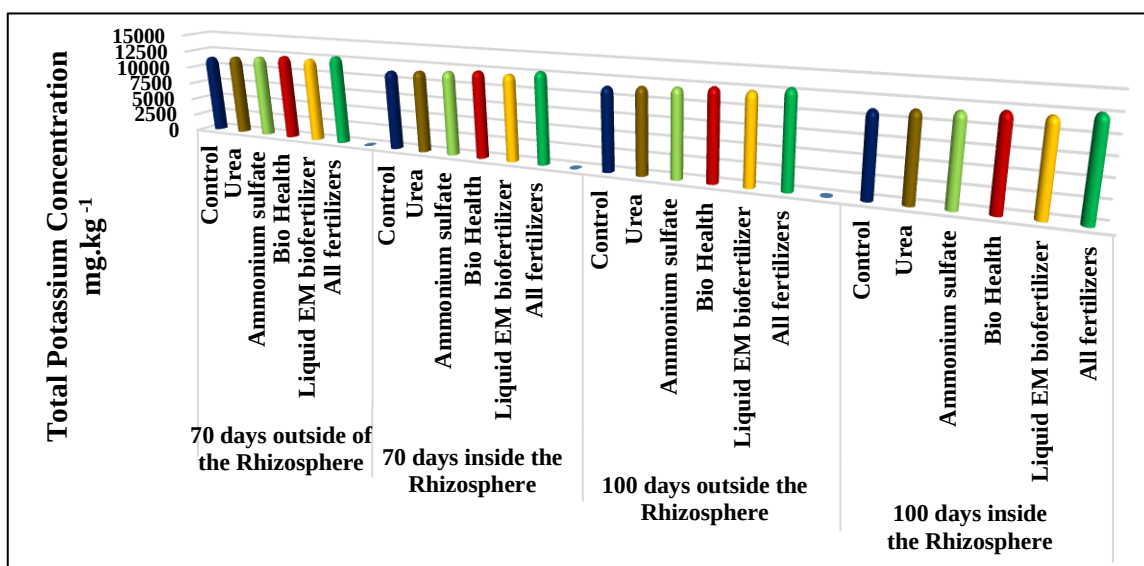


Figure 2. Impact of fertilizer type on total potassium concentration in both rhizosphere and bulk soil at 70- and 100-days post-planting (mg kg⁻¹).

Figure 2 and the data in Tables 4 and 5 indicate the total potassium concentration consistently decreased across the measurement periods of 70 and 100 days in the rhizosphere soil across all treatments, with a significant loss observed at 100 days.

Conclusion

This study investigated the impact of biofertilizers and mineral fertilizers on the availability of potassium in silty clay soils cultivated with yellow maize. The treatments included mineral fertilizers (urea and ammonium sulfate), biofertilizers (BioHealth and liquid EM), and combinations of both. Potassium levels were measured 70 and 100 days after planting, both within and outside the rhizosphere. The results showed that combining biofertilizers with mineral fertilizers gave the highest levels of available and total potassium, whereas BioHealth alone performed best among the single treatments. Potassium availability was consistently lower in the rhizosphere than in the bulk soil.

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Effect of Organic and Bio-Fertilizer Type on Available Phosphorus Concentration in Soil Cultivated with Maize (*Zea mays* L.)

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Abstract. To study the effect of organic and biological fertilizers on the concentration of available phosphorus in the rhizosphere of yellow corn, an experiment was conducted in an agricultural field during the autumn season 2024-2025. The experimental treatments included vermicompost, compost, tornado fertilizer, and biofertilizer represented by seaweed extract, in addition to the fertilizer combination (25% of the fertilizer recommendation for all fertilizers). Yellow corn (*Zea mays* L.) seeds were planted. Available phosphorus in the soil was estimated during the plant growth periods (70, 100) days from planting. The results showed that the addition of organic fertilizer, biofertilizer, and the fertilizer combination significantly increased the concentration of available phosphorus in and outside the rhizosphere soil during the studied time periods (70, 100) days from planting. The fertilizer combination achieved the highest concentration of available phosphorus, reaching (15.85, 16.31) mg. kg⁻¹ in the rhizosphere soil and (17.06, 19.75) mg. kg⁻¹ outside the rhizosphere compared to the control treatment which achieved the lowest concentration of available phosphorus and reached (3.47, 4.71) mg. kg⁻¹ in the rhizosphere soil while in the extra-rhizosphere soil it reached (5.31, 6.10) respectively. The results also indicate that the addition of Tornado significantly increased the phosphorus concentration and reached (12.42, 11.44) mg. kg⁻¹ in the rhizosphere soil and (13.68, 15.21) mg. kg⁻¹ outside the rhizosphere during the studied time periods (70, 100) days from planting for the single fertilizer, which indicates its significant role in supplying phosphorus to the plant. The fertilizers took the following sequence in their ability to supply phosphorus: fertilizer mixture > tornado > seaweed extract > vermicompost > compost.

Keywords. Organic fertilizer, biofertilizer, available phosphorus, compost, seaweed extract.

1. Introduction

Sustainable agriculture is essential because it provides solutions to the current issues related to traditional agriculture. Soil is a non-renewable resource and sustainable agriculture is committed to protecting and preserving its health. In addition, sustainable agriculture addresses most public concerns and reforms in industrial practices, such as reducing the use of antibiotics, as production and processing conditions can negatively affect human and animal health [1]. The use of

chemical fertilizers and industrial pesticides damages soil health and negatively affects ecosystems by polluting water and the environment. To protect future generations from these harmful effects, we must transition to organic farming, and restore and maintain soil fertility using sustainable methods [2].

Organic fertilization also provides a way to recycle organic waste, such as food scraps and agricultural waste, keeping it out of landfills and reducing greenhouse gas emissions [3]. Worm compost is a good soil conditioner owing to its chemical and physical properties. It contributes to increasing the soil's water retention capacity, which increases its moisture content and reduces water runoff [4].

Vermicompost is a rich source of enzymes, humic substances, beneficial microorganisms, and some compounds that are important for plant growth, thus contributing to the improvement of soil fertility [5,6]. Worm castings increase soil reactivity because of their high content of major cations and their buffering capacity, as some nutrients, such as phosphorus, become more readily available to plants. They also help to reduce some elements such as aluminum, manganese, and iron [7,8].

The use of biofertilizers is a promising technique for sustainable farming systems because of the rapid decline in phosphate reserves and the need to use available nitrogen more efficiently. Biofertilizers are natural materials containing live microorganisms, such as algae, fungi, and bacteria, or their byproducts. These materials are used in agricultural practices by adding them to soil, seeds, or plant surfaces to stimulate plant growth and improve soil fertility [9].

Seaweed extract is rich in vitamins that contribute to the activation of photosynthesis, participate in electron transfer, regulate oxidation and reduction processes, and activate the formation of natural auxins within plants [10]. Seaweed extracts are effective and economical plant growth-stimulants that improve plant growth and development. They enhance crop quality and quantity by increasing the efficiency of nutrient and water use, even under harsh environmental conditions [11]. The integration of biofertilizers into agricultural practices provides an effective means of enhancing the microbial status of the soil and facilitating the activity of microorganisms in natural soils, which affects food availability and degradation of natural resources [12].

Phosphorus (P) is one of the most important nutrients in plants [13]. Phosphorus is the second most important nutrient for crops after nitrogen, with the total phosphorus content in the soil ranging from approximately 0.02% to 0.15%. Most of the phosphorus in the soil is not available to plants and is often a limiting nutrient for crop production [14]. In soil solutions, phosphorus is mainly present as an anion ($\text{H}_2\text{PO}_4^{-1}$) at pH values between 3.0 and 7.0, and as an anion (HPO_4^{-2}) at pH values above 7.2 [15-17].

Plants absorb phosphate in the form of monobasic ($\text{H}_2\text{PO}_4^{-1}$) or dibasic (HPO_4^{-2}) ions. Phosphorus is essential for the formation of seeds that contain high levels of phosphorus [18]. In contrast, phosphorus deficiency causes a reduction in plant size and growth [19].

Yellow corn (*Zea mays* L.) is one of the world's most important grain crops [20]. It is preferred over other crops, because it can be grown in almost all seasons and locations. It is a strategic grain crop for global production in many countries, including Iraq. It is used in energy and biofuel production and in the feed industry and is characterized by high nutritional value, as starch and oil are extracted from it. It contains proteins and vitamins A, B1, and B2 [21].

Based on the above, this study aimed to examine the effect of organic matter and biofertilization on phosphorus availability in soil planted with yellow corn.

2. Materials and Methods

2.1. Experiment Location

The experiment was conducted in an agricultural field in Diwaniya, Iraq, near the Faculty of Agriculture, University of Qadisiyah, at latitude N 31.962823 and longitude E 44.779969, during the autumn season of 2024. The soil was silt clay loam and the selected field area was 260 m². Agricultural operations were conducted on the soil, including plowing, smoothing, and leveling the estimated area for the experiment. Three main furrows were created across the field, with secondary furrows for each plot, and the field soil was divided into three sectors, each with six plots measuring 3 m × 3 m as an experimental unit, leaving a distance of 1 m between the experimental units. The field was fertilized with all treatments before planting, with the exception of algae extract, which was sprayed on the plants 30 d after germination. Yellow corn (*Zea mays* L.) of the local variety was planted in rows at a distance of 60 cm between rows and 25 cm between holes, with four rows in each plot. Three seeds were placed in each hole, and after two weeks, the plants were thinned to one plant per hole. All crop maintenance operations were performed, including irrigation and weed removal, as needed. Weeding and hoeing were performed when the weeds and bushes were observed. The experimental units were irrigated with equal amounts of water according to the needs of the plants.

2.2. Studied Properties

Soil fractions were estimated using the hydrometer method, as described in [22].

The apparent density was estimated using the core sample method, as described in [22].

Soil reactivity was measured using a pH meter in a soil-water extract (1:1), according to the method described in [23].

- Organic matter was estimated using the wet digestion method with 1N K₂Cr₂O₇ according to the Walkley and Black method [22].
- The exchange capacity of positive ions was estimated using ammonium acetate and sodium acetate, according to [22].
- Carbonate minerals were estimated by titration with (N1) HCL and back titration with (N1) NaOH according to the method described in [23].
- Available nitrogen: The available ammonium ions in the soil were estimated by extraction with potassium chloride solution (2 molar) and using magnesium oxide (MgO), and it was distilled after evaporation using a Micro Kjeldahl device. After extracting the ammonium ion, the nitrate ion was reduced using Devarda alloy and distilled using a Micro Kjeldahl device, according to the method described by Bremner (1965) [23].
- Available phosphorus in the soil was extracted during the plant growth periods (70 and 100 days) after planting using sodium bicarbonate (0.05M NaHCO₃) at a pH of 8.5, according to the Olsen method. The color was developed with ascorbic acid and ammonium molybdate and then measured using a spectrophotometer at a wavelength of 882 nm according to [23].
- Potassium was extracted with ammonium acetate solution (1 standard) and estimated using an AFP100 flame photometer according to the method described in [23].
- Magnesium and calcium were estimated by titration with ferric acid (Na₂-EDTA) using the Eriochrome Black T reagent, and then calcium was subtracted from the sum of magnesium and calcium according to the method described by Lanyon and Heald [23].

- Sodium was estimated using a flame photometer, according to the method described in [24].
- Potassium dissolved in soil extract: water (1-1) was estimated using a flame photometer according to the method proposed by Knudsen et al. [23].
- Chlorine was estimated by titration with silver nitrate (AgNO_3) at a concentration of N 0.01 in the presence of potassium chromate, according to the method described in [24].
- Carbonate was estimated by titration with concentrated sulfuric acid (H_2SO_4) at a concentration of N 0.01 using phenolphthalein indicator, according to the method described in [24]. Bicarbonates were determined by titration with concentrated sulfuric acid (H_2SO_4) at a concentration of (N0.01) in the presence of a methyl orange indicator, according to the method described in [24].
- Sulfates were estimated by precipitation in the form of barium sulfate according to a previously described method [22].

Table 1. Some chemical and physical properties of the study soil.

Parameters	Value	Unit
pH 1:1	7.69	-----
EC 1:1	2.53	ds m^{-1}
CEC	22.13	$\text{C mol}_c \text{ kg}^{-1} \text{ soil}$
Cationic dissolved ions	15.24	Ca^{+2}
	10.43	Mg^{+2}
	22.20	Na^{+1}
	1.58	K^{+1}
Anionic dissolved ion	23.00	Cl^{-1}
	19.00	HCO_3^{-1}
	15.10	SO_4^{-2}
	Nil	CO_3^{-2}
Carbonate	255.00	
Organic matter	4.65	$\text{g Kg}^{-1} \text{ soil}$
Available phosphorus	13.1	
Available potassium	137.00	
Available nitrogen	26.11	$\text{mg kg}^{-1} \text{ soil}$
Soil texture	Silty clay mixture	
Bulk density	1.33	Mg m^{-3}
Particle size distribution	sand	217.00
	silt	368.00
	clay	415.00

2.3. Experimental Design

The experiment was designed according to a Randomized Complete Block Design (RCBD). Six experimental treatments, including a control treatment, were used with three replicates for each treatment. The treatments were randomly distributed across the experimental units, bringing the total number of units to 18. The experiment included the following treatments.

Control treatment: no fertilizer was added.

- Compost fertilizer was added at a single level at a concentration of 25 kg. h^{-1} .
- Vermicompost fertilizer was added at a single level and at a concentration of 25 kg. h^{-1} .
- TORNADO fertilizer was added at a single level and at a concentration of 7 kg. h^{-1} .
- Seaweed extract fertilizer was added at a single level and at a concentration of $1.5 \text{ liters h}^{-1}$.

- Adding all fertilizers: The addition was done at one level and at a quarter of the recommended concentration of the above fertilizers (25% vermicompost fertilizer, 25% compost fertilizer, 25% seaweed extract fertilizer, and 25% Tornado fertilizer).

Table 2. Some characteristics of fertilizers used in the study.

Fertilizer	Components
Compost (Hortimed Company – Made in EUROPEAN UNION)	Organic fertilizer composed of decomposed animal and plant residues, rich in essential nutrients such as nitrogen (N), phosphorus (P), and potassium (K), along with micronutrients. It is applied to the soil before planting or around established plants. This fertilizer improves soil structure, enhances water-holding capacity and aeration, and increases soil fertility, providing a slow-release and long-term sustainable effect.
Vermicompost (HUMOBACTER-A_ Made in BLUEFIELD Company_ IRAQ)	Organic fertilizer produced from plant and animal residues decomposed by earthworms. This fertilizer is rich in macronutrients (N, P, K) and has a high concentration of micronutrients. It can be applied in solid or liquid form. Vermicompost significantly improves soil structure, drainage, and porosity, promotes root development, enhances fruit quality, and increases plant resistance to pests and diseases.
Tornado (Tornado fertilizer_ made in EGYPT)	Organic granular fertilizer primarily composed of humic acid, fulvic acid, and potassium. It can be applied directly to the soil in solid form or dissolved in water. This fertilizer rapidly enhances soil fertility and plant nutrition, providing quick and effective benefits to both soil and crops.
Seaweed extract (Greenhas Group _ made in ITALY)	A liquid biofertilizer extracted from marine algae, containing approximately 2.3% organic nitrogen, 16.1% organic carbon, and 32.2% organic matter. It is applied either through irrigation water or as a foliar spray. Rich in natural hormones, growth stimulants, vitamins, and amino acids, this biofertilizer rapidly enhances flowering and fruit set, improves overall plant quality, and strengthens resistance to environmental stresses, delivering an immediate and fast-acting boost to plant growth.

2.4. Statistical Analysis

The results were analyzed statistically using analysis of variance (ANOVA) within a randomized complete block design (R.C.B.D). The least significant difference (LSD) was calculated to test the difference between treatments at a 5% probability level [25]. using the GENSTAT v12 program.

3. Results and Discussion

3.1. Effect of Organic and Biological Fertilizers on the Availability of Available Phosphorus (mg. kg^{-1}) for 70 Days After Planting

Table 3 shows the effect of adding organic and biological fertilizers on the concentration of available phosphorus in the rhizosphere and extra-rhizosphere soils 70 d after planting. At a significance level of 0.05, the results of the statistical analysis showed significant differences in the concentration of available phosphorus. All treatments outperformed the control treatment. The highest phosphorus concentration was in the fertilizer combination treatment, reaching (15.85, 17.06) mg. kg^{-1} in the rhizosphere and extra-rhizosphere soils, respectively, with increases of 356.77% and 221.28%. The lowest concentration of available phosphorus was observed in the control treatment, reaching (3.47, 5.31) mg. kg^{-1} in the rhizosphere and extra-rhizosphere soils, respectively.

Table 3 shows the phosphorus concentrations in the individual fertilization treatments. The available phosphorus concentration in the organic fertilizer treatments, represented by Tornado fertilizer, reached (11.44, 13.68) mg.kg^{-1} in the rhizosphere and extra-rhizosphere soil, respectively. The phosphorus concentration soil, respectively; compost fertilizer reached (10.72, 11.65) mg.kg^{-1} in the rhizosphere and extra-rhizosphere soil, respectively; and compost fertilizer (8.68, 9.18) mg.kg^{-1} , respectively. The available phosphorus concentration in the seaweed extract treatment reached (10.92, 11.68) mg.kg^{-1} in the rhizosphere and extra-rhizosphere soil, respectively. The increased available phosphorus concentration was attributed to the mineralization of organic matter containing phosphorus. The increase in phosphorus concentrations also indicates that early treatments are very effective in improving phosphorus availability in the soil, which is consistent with [26,27,28].

Table 3. Effect of organic and biological fertilizer on the concentration of available phosphorus (mg.kg^{-1}) in rhizosphere soil and non-rhizosphere soil after 70 days after planting.

Treatment	Rhizosphere	Bulk soil
Control	3.47	5.31
Compost	8.68	9.18
Vermicompost	10.72	11.65
Tornado	11.44	13.68
Seaweed extract	10.92	11.68
Mix	15.85	17.06
L.S.D 0.05	3.31	3.3919

3.2. Effect of Organic and Biological Fertilizers on the Concentration of Available Phosphorus (mg.kg^{-1}) for 100 Days After Planting

Table 4 shows the effect of adding organic and biological fertilizers on the concentration of available phosphorus in the rhizosphere and extra-rhizosphere soils 100 d after planting. At a significance level of 0.05, the results of the statistical analysis showed significant differences in the concentration of available phosphorus. All treatments outperformed the control treatment. The highest phosphorus concentration was observed in the fertilizer combination treatment, which was (16.31, 19.75) mg.kg^{-1} in the rhizosphere and extra-rhizosphere soils, respectively, with increases of 246.30% and 223.77%, respectively, while the lowest phosphorus concentration was in the control treatment, which was (4.71, 6.10) mg.kg^{-1} .

Table 4 shows the phosphorus concentrations in the individual fertilization treatments, all of which showed significant differences. The phosphorus concentration in the tornado fertilizer reached (12.42, 15.21) mg.kg^{-1} in the rhizosphere and outside, respectively, whereas the phosphorus concentration in the vermicompost fertilizer reached (11.06, 13.74) mg.kg^{-1} in the rhizosphere and outside, respectively, while its concentration in the compost fertilizer reached (10.12, 11.64) mg.kg^{-1} in the rhizosphere and outside, respectively. As for the seaweed extract treatment, the phosphorus concentrations reached (12.26, 12.79) mg.kg^{-1} in the rhizosphere and outside, respectively. The significant increase in available phosphorus concentrations in all fertilization treatments was attributed to the significant development of roots with the progress of the growth stage, which in turn increased the surface area of absorption. Root development enhances the ability of plants to access phosphorus in the soil [29]. Corn root exudates play a major role in the secretion of organic acids and sugars, which in turn dissolve phosphorus from insoluble compounds. In other words, exudates enhance phosphorus availability [30]. Microbial

activity also plays an important role in phosphorus availability because of its interaction with plant roots, which can analyze and decompose organic matter [31,32].

Table 4. Effect of organic and biological fertilizer on the concentration of available phosphorus (mg. kg^{-1}) in the rhizosphere soil and non-rhizosphere soil 100 days after planting.

Treatment	Rhizosphere	Bulk soil
Control	4.71	6.10
Compost	10.12	11.64
Vermicompost	11.06	13.74
Tornado	12.42	15.21
Seaweed extract	12.26	12.79
Mix	16.31	19.75
L.S.D 0.05	3.2386	2.0580

It is noted from the results shown in Table 3 and 4 and Figure 1 that the concentration of available phosphorus continued to increase with measurement periods of (70-100) days in and outside the rhizosphere soil for all treatments. This is because the increased growth of yellow corn causes significant development of roots in the soil, which can access phosphorus in distant areas that contain higher levels of phosphorus. Microscopic organisms, such as fungi and bacteria, also contribute to enhancing the availability of phosphorus by performing the processes of decomposing and breaking down organic compounds, thereby increasing the availability of phosphorus [29]. A low C:P ratio stimulates microorganisms to decompose organic matter and absorb nutrients, which increases the content of available phosphorus in the soil [33]. Available phosphorus differed from the other elements studied in that its concentration increased with increasing growth duration both inside and outside the rhizosphere. This may be due to the decreased plant requirements during the second 100 d after planting. The sequence of treatments for phosphorus supply inside and outside the rhizosphere is as follows:

Fertilizer combination > Tornado > Algae extract > Vermicompost > Compost.

Ready phosphorus differed from total phosphorus in that the combination of fertilizer and biofertilizers outperformed organic fertilizers.

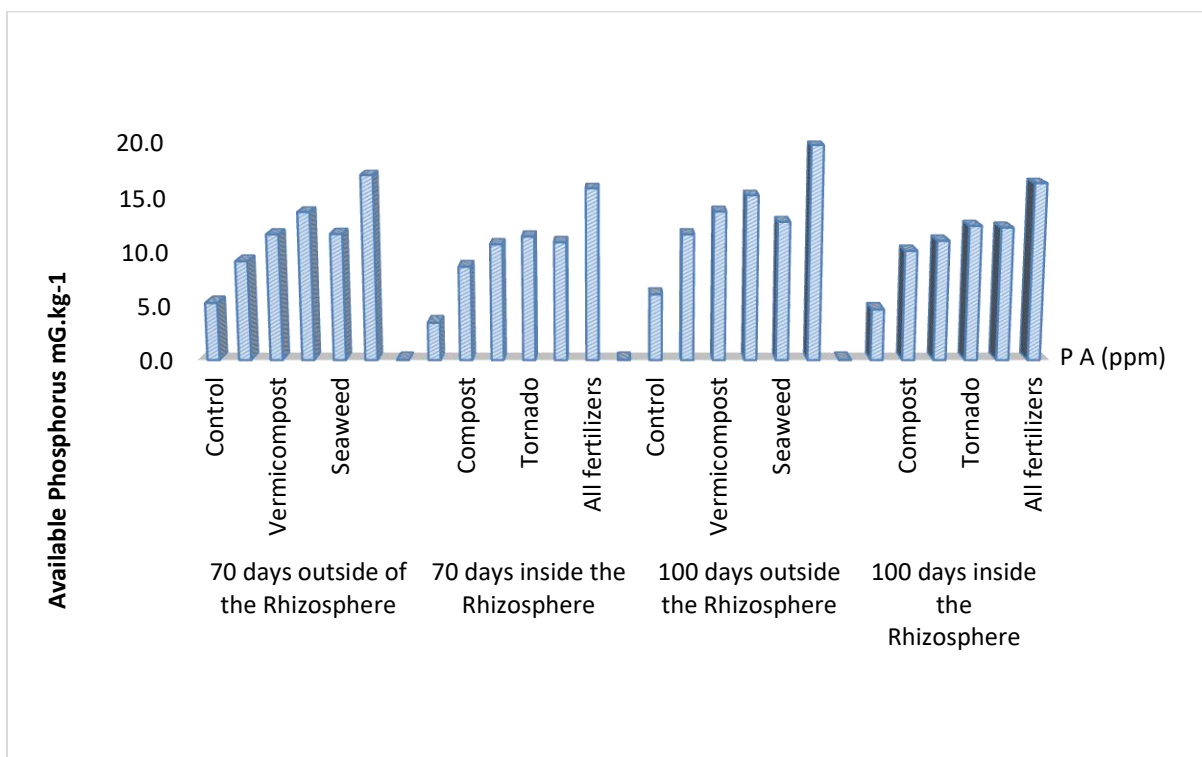


Figure 1. The effect of organic and biological fertilizer on the concentration of available phosphorus (mg. kg^{-1}) in rhizosphere soil and non-rhizosphere soil.

Conclusion

The application of organic and biofertilizers significantly enhanced the concentration of available phosphorus in both rhizosphere and non-rhizosphere soils of yellow corn compared with the unfertilized control. Among all treatments, the fertilizer mixture (25% of the recommended rate for each fertilizer) produced the highest phosphorus availability at both 70 and 100 days after planting, followed by tornado, seaweed extract, vermicompost, and compost. These results demonstrate that integrating different organic fertilizers and biofertilizers, particularly the combined fertilizer mixture, can effectively improve soil phosphorus availability and support sustainable phosphorus management in maize cultivation.

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Evaluation of Lipid Content in Select Microalgae Species and Their Potential Application as Carriers for Oral Drug Delivery Systems in Vitro

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Abstract. Microalgae are emerging as potent platforms for lipid-based oral drug delivery systems (DDSs) due to their natural biocompatibility, lipid richness, and versatility. This study aimed to evaluate the lipid productivity, encapsulation efficiency, and drug delivery potential of five microalgal species: *Chlorella vulgaris*, *Dunaliella salina*, *Microchloropsis gaditana*, *Haematococcus lacustris* and *Limnospira platensis*. Cultures were grown under semi-controlled photobioreactor conditions, and lipids were extracted via the Bligh and Dyer method. Analytical characterization was performed using GC-MS, FTIR, and ¹H/¹³C NMR. Drug-loaded lipid nanoparticles, incorporating curcumin and ibuprofen, were formulated using thin-film hydration and emulsification methods. Results demonstrated that *C. vulgaris* had the highest biomass productivity (11,000 mg/L/day) and lipid content (52% DW), along with the top encapsulation efficiency (82%) and drug permeability (~0.204 µg/mL). *H. lacustris* followed with 10,000 mg/L/day biomass, 40% lipid content, and 80% efficiency and drug permeability (~0.200 µg/mL), enhanced by astaxanthin-rich vesicles under CO₂/light stress. *M. gaditana* exhibited EPA-rich lipids with moderate productivity (4,500 mg/L/day), 75% encapsulation and drug permeability (~0.187 µg/mL). In contrast, *D. salina* and *L. platensis* showed lower lipid yields (30% and 20%, respectively) and reduced encapsulation (68% and 60%) and drug permeability (~0.170 and ~0.150 µg/mL) respectively. Conclusively *C. vulgaris* and *H. lacustris* were identified as optimal candidates for lipid-based oral DDSs.

Keywords. Microalgae, Lipid extraction, Oral drug delivery, Nanoparticles, Encapsulation efficiency.

1. Introduction

In recent years, microalgae have drawn increasing attention because of their remarkable biochemical diversity and potential for many biotechnologically applications [1]. Despite the large range of bioactive molecules produced by microalgae, lipids are an important class of metabolites, not only for their applications in biofuels and nutritional formulations [2], but also in the pharmaceutical industry as drug carriers, since their amphiphilic character is useful in drug delivery systems [3]. The increasing demand for biocompatible and biodegradable oral drug carriers that can offer stability and controlled release has led to the exploration of algal lipids [4]. Oral administration is the preferred procedure because it is non-invasive and self-administered, although it is limited by enzymatic degradation and poor GI absorption [5]. Consequently, a considerable number of bioactives compounds have poor bioavailability, which in turn restricts

their therapeutic efficacy, particularly in the case of peptides, proteins, and hydrophobic drugs [6]. Microalgae-based lipids for drug delivery systems (DDSs) are promising because of their ability to improve drug solubility, resistance to enzymatic digestion, and controlled or site-specific release [7]. These oils, which contain high amounts of polyunsaturated fatty acids (PUFAs), form stable emulsions or vesicles suitable for entrapping labile drugs [8]. However, microalgal lipid content, profiles are diverse among species, indicating that complete biochemical characterization is necessary to highlighted the best candidate strains [9]. The bioactive fractions extracted from some microalgae species and their evaluation as oral DDSs have shown potential in vitro [10]. Nanosized carriers have been developed for oral applications, including polymeric nanoparticles, liposomes, and plant- or algal-origin biomimetic vesicles [11]. Creative systems including floating (buoyant in gastric fluid) and mucoadhesive DDSs (which adhere to mucosal linings), extend the GI residence time and are promising for enhancing absorption [12]. This approach, in combination with the inherent lipid moieties of microalgae, may provide a synergistic strategy for the development of next- generation oral drug formulations [13].

Mucoadhesive DDSs using algal biopolymers aim to enhance biodegradability and non-immunogenicity, thereby improving systemic uptake and site-specific release [14,15]. Microalgae are autotrophic photosynthetic microorganisms with a high nutrient content including proteins, carbohydrates, lipids, vitamins, and bioactive compounds with antioxidant, immunostimulant, antibacterial, and neuroprotective activities [16,17]. Prokaryotic cyanobacteria (e.g., *L. platensis*, *Nostoc commune*) and eukaryotic green algae (e.g., *C. vulgaris*) synthesize a number of lipids, including PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have cardioprotective and neuroprotective properties [16]. The lipid content in microalgae typically ranges between 20% and 50% of the dry weight [15]. Lipid extraction is often solvent-based; for example, using the Bligh and Dyer method (up to 84% recovery) [18] or supercritical fluid extraction [19]. The analysis of triacylglycerols in FAMES can be performed via gas chromatography (GC), where transesterification is used to convert them into FAMES, followed by GC-MS for lipid profile characterization [20]. NMR (¹H and ¹³C-NOESY-ERIC referencing) has also been used for further structural interpretation [21]. Mass spectrometry methods including ESI-IT-Orbitrap and MALDI-TOF further support lipid characterization [22]. Selected strains such as *Nannochloropsis oculata* (17.5% lipid content), *Phaeodactylum tricornutum*, *Schizochytrium limacinum*, and *C. vulgaris* showed high bioactivity and lipid content, making them suitable for DDS development [14]. Culture conditions, such as nutrient limitation and light intensity, significantly influence lipid accumulation, and fatty acid synthesis enzymes are upregulated in response to induced stress [23]. A Transwell Caco-2 cell assay in vitro demonstrated that aNPs from *S. obliquus* and *L. clathratulus* significantly increased drug permeation (0.204 µg/mL and 0.14 µg/mL, respectively) compared to LipoX (0.036 µg/mL) and free drug (0.008 µg/mL) [5]. Endocytosis inhibitors have confirmed the involvement of multiple cellular internalization pathways. Nutraceutical microalgal products include functional foods fortified with PUFAs, carotenoids, and antioxidants, which enhance cardiovascular, neurological, and metabolic health [16]. In the cosmetics industry, species such as *Chlorella*, *Spirulina*, and *Dunaliella* are used for skin-benefiting bioactivity [16]. Despite the challenges in scalability, regulatory compliance, and formulation stability, the potential of microalgae for drug delivery is considerable. Up-scaling of biomass production has been facilitated by processes such as the Smash-Open-Filter (SOF), and economic feasibility has been improved by solvent recovery methods and green extraction technologies. These advances support the viability of microalgae as sustainable sources of active compounds for pharmaceutical, nutraceutical, and cosmetic industries [24,25].

1.1. Contribution of Microalgae in Lipid Production

Microalgae such as *C. vulgaris*, *D. salina*, *N.gaditana*, and *H.lacustris* have been reported to contain high levels of lipids, mainly triglycerides, phospholipids, and PUFAs. Lipid composition varies by species and is highly influenced by light conditions, nutrient supply, and growth phases. Vieira et al. demonstrated how these factors influence lipid yield and fatty acid profiles, thereby affecting their suitability for drug delivery [26].

1.2. Extraction and Characterization Techniques

Efficient recovery and characterization of microalgal lipids are critical for both research and commercial viability of drug delivery applications. The Bligh and Dyer method remains widely used because of its high yield across biomass types, while newer methods such as supercritical CO₂ extraction and pressurized liquid extraction offer greater selectivity and environmental benefits. Analytical methods including GC-MS, FTIR, and TLC are essential for profiling fatty acids and identifying bioactives [27–29].

1.3. Lipid Production of Microalgae for Oral Drug Delivery

Lipid-based delivery systems are widely used to address the poor solubility and gastrointestinal (GI) degradation of many drugs. Microalgal lipids can be emulsified and encapsulated to improve their solubility, protect labile compounds, and allow controlled release.. synthesized algae-derived lipid nanoparticles that successfully crossed in vitro intestinal barriers and showed promise for oral delivery [5].

1.4. Advantages and Limitations

Microalgae-derived lipid carriers are biocompatible, biodegradable, and naturally antioxidant-rich, which makes them ideal for protecting drugs from oxidative stress. Their capacity to carry both hydrophilic and hydrophobic drugs is varied. However, challenges regarding scalability, batch consistency, formulation stability, and the need for in vivo toxicity and safety studies [4,7,13]. Microalgae are promising candidates for oral drug delivery systems owing to their rich lipid content, biocompatibility, and bioactive properties. However, challenges related to extraction efficiency, characterization, and clinical translation remain. Therefore, this study aimed to evaluate lipid yields from selected microalgae species and assess their potential as carriers for oral drug delivery.

2. Materials and Methods

Lab Approval Statement: The operations of laboratories in the Department of Microbiology and Clinical Laboratory Sciences, located at the College of Pharmacy, University of Basrah, have been reviewed and approved for conducting experimental research in accordance with relevant scientific and safety standards, including applicable standards such as dosage form, biomass and lipid contents (Good Laboratory Practice) or ISO 17025], as of from 1 January, 2025 to 1 of April 2025.

2.1. Microalgae Species Selection

Pure axenic cultures of algae used in the current study were obtained from the Advanced Environmental Laboratory in the Department of biology, College of Education, University of Al-Qadisiyah. Five microalgae were selected as the positive controls (Figure 1), whose high lipid content and application in the pharmaceutical industry are highly recognized: *Chlorella vulgaris* Beijerinck 1890, *Dunaliella salina* (Dunal) Teodoresco 1905, *Microchloropsis gaditana* (L.M.Lubián) M,W.Fawley, I.Jameson & K.P.Fawley 2015, *Haematococcus lacustris* (Girod-

Chantrans) *Rostafinski* 1875, and *Limnospira platensis* (Gomont) K.R.S.Santos & Hentschke 2023. [25,26]. The identification for algae in the present study was done by Inverted Microscope microscope and by the accreditation on the taxonomic keys [30].

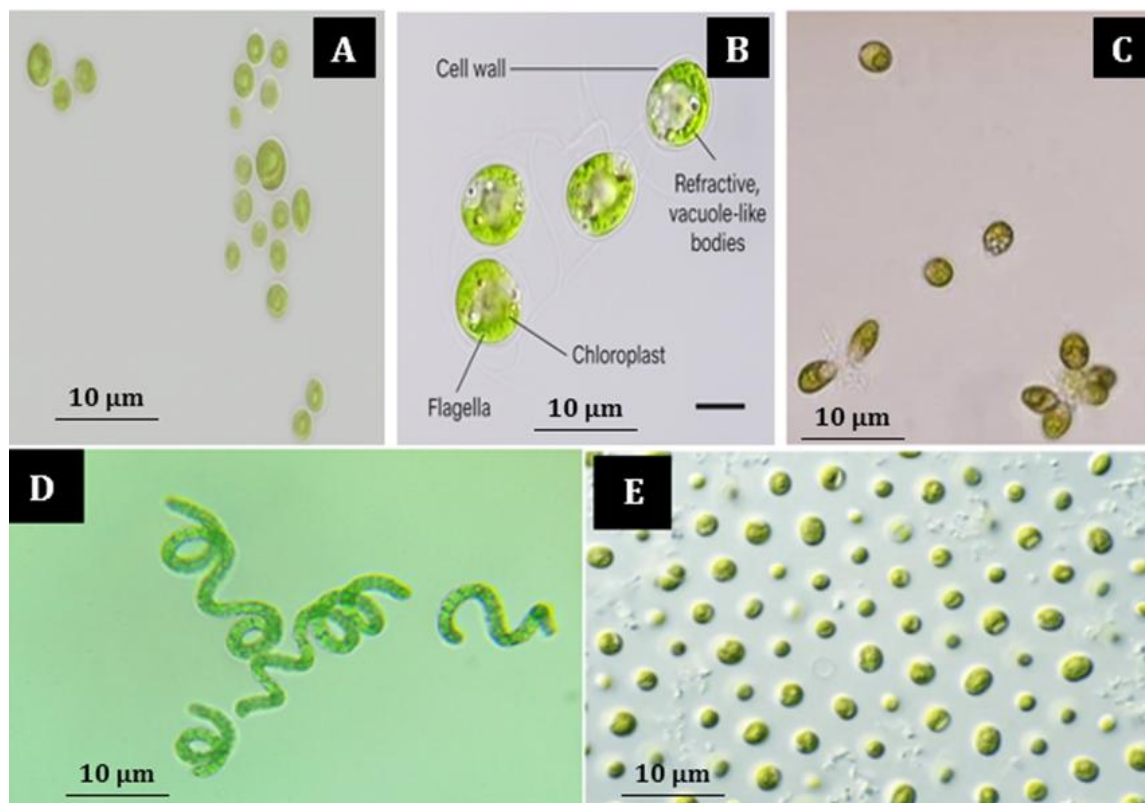


Figure 1. (A) *C. vulgaris* , (B) *H. lacustris* , (C) *D. salina* (D) *L. platensis* , (E) *N. gaditana* under light microscope X40.

2.2. Cultivation Conditions

To ensure that the isolates were free of bacteria and fungi, a sample of the algal isolate was planted on the solid culture media Nutrient agar for testing bacteria and was incubated at 37°C for 72 hours to ensure its purity [31]. Microalgae were grown under controlled photoautotrophic conditions in BG-11 (for freshwater species) and F/2 (for marine species). Media cultures were maintained under a light intensity of $100 \mu\text{mol photons m}^{-2}\text{s}^{-1}$, at a temperature of $25 \pm 2^\circ\text{C}$, and aerated with sterile air enriched with 1–2% CO_2 to promote optimal growth and lipid content [27,28].

2.3. Lipid Extraction

Lipid extraction was primarily performed using the Bligh and Dyer method, which efficiently extracts total lipids from microalgal biomass using a chloroform–methanol–water system [29]. For comparison, supercritical CO_2 extraction was used to select samples to evaluate the feasibility of obtaining cleaner lipid fractions with reduced solvent residues [32].

2.4. Lipid Characterization

The extracted lipids were analyzed using several biochemical, including techniques Fourier Transform Infrared Spectroscopy (FTIR) for functional group screening, Thin Layer Chromatography (TLC) for lipid class separation, and gas chromatography–mass spectrometry (GC-MS) to study the fatty acid methyl ester (FAME) profiles. These analytical approaches enable the accurate identification of lipid classes and detailed fatty acid profiles [32,27].

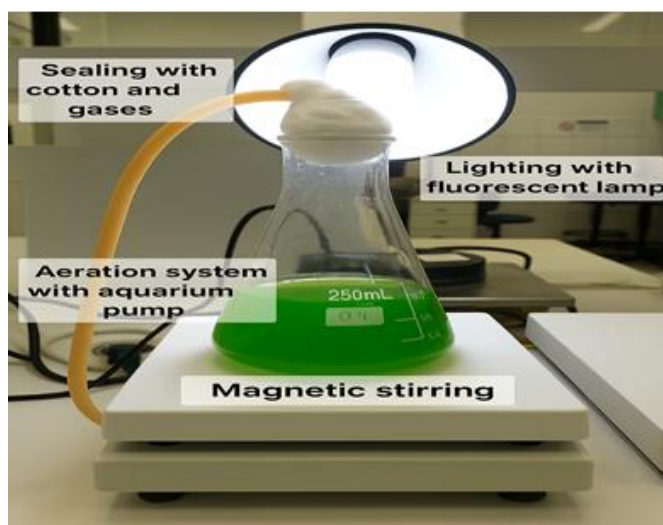


Figure 2. Laboratory setup for microalgae bioreduction under controlled conditions.

The system consisted of a 250 mL Erlenmeyer flask containing the microalgal culture, sealed with cotton to allow gas exchange while preventing contamination. Aeration was provided by an aquarium pump to maintain oxygenation, and magnetic stirring ensured the homogeneous mixing of the medium. A fluorescent lamp provides consistent lighting to support photosynthetic activity, simulating the daylight conditions required for optimal microalgal growth and biocatalytic activity.



Figure 3. *C. vulgaris* prepared solution.

2.5. Preparation of Drug Delivery Vehicle

Two types of drug delivery systems were prepared using the extracted microalgae lipids: liposomes using the thin-film hydration method, and lipid nanoparticles using emulsification or solvent evaporation methods. Curcumin was used as a model hydrophobic drug because of its therapeutic relevance, while Ibuprofen was used to assess the encapsulation and release behavior of a commonly used non-steroidal anti-inflammatory drug [34,35].

2.6. In Vitro Evaluation

- Step 1: Setup of the bioreduction reaction medium with seawater saline solution, Conway medium, and acetophenone
- Step 2: Homogenization of the reaction mixture using a magnetic stirrer to ensure uniform distribution of the components.
- Step 3: Inoculation of fresh microalgae into saturated culture medium for biomass buildup: The bioreduction reaction medium includes microalgae.
- Step 4: The bioreduction phase begins, in which microalgae act as biocatalysts in the presence of acetophenone under controlled conditions. [34,35].

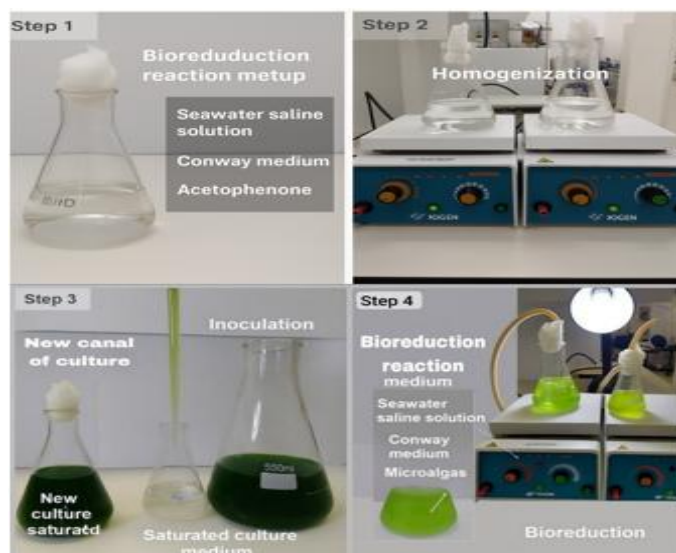


Figure 4. Stepwise process for bioreduction reaction using microalgae cultures.

2.7. Techniques Used

This process involves the selection of microalgae species, followed by cultivation and harvesting, lipid extraction, formulation of drug delivery systems, and subsequent in vitro evaluation of therapeutic potential. Infrared Spectroscopy (FTIR) and thin layer- chromatography (TLC).

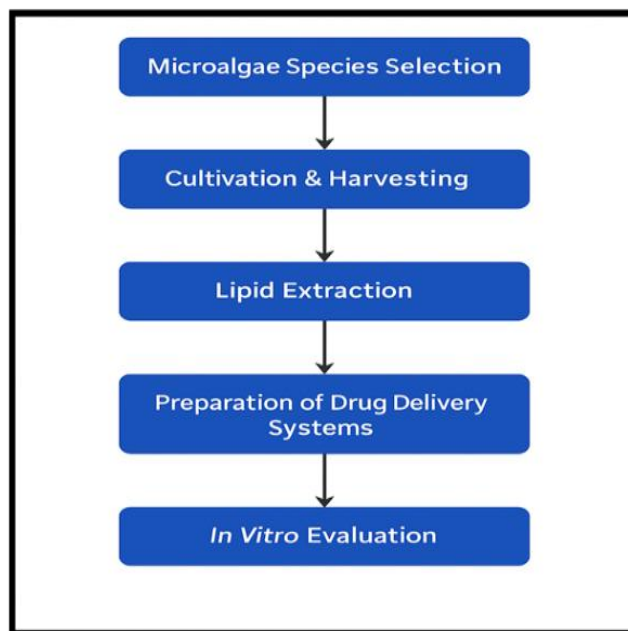


Figure 5. Workflow illustrating the development of microalgae-derived drug delivery systems.

2.8. Statistical Analysis

All statistical analyses were conducted using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Experimental data are expressed as mean $\pm$ standard deviation (SD) based on triplicate measurements. One-way Analysis of Variance (ANOVA) was used to compare biomass productivity, lipid content, and drug encapsulation efficiency across the five microalgae species. Tukey's post-hoc test was applied for pairwise comparisons when significant differences were observed ($p < 0.05$). Correlation coefficients (Pearson's r) were calculated to assess the relationship between the lipid content and encapsulation efficiency. Significance levels were reported as follows: ($p < 0.05$), ($p < 0.01$), and ($p < 0.001$). These statistical tools allowed the validation of observed trends and ensured the robust interpretation of interspecies variability in lipid and drug delivery performance.

3. Results

3.1. Microalgae Biomass and Lipid Productivity

Table 1 and Figure 6 compares the five microalgae species based on biomass production, growth characteristics, and lipid productivity. *M. gaditana* demonstrated the highest lipid harvesting efficiency, with the highest lipid productivity (0.108 g/L/day) and biomass (0.458 g/L). *C. vulgaris* exhibited reliable growth under semi-continuous conditions, yielding moderate lipid productivity (0.011 g/L/day) and good biomass (0.263 g/L). *H. lacustris* shows high biomass (0.410 g/L) when cultured under CO₂/light stress, although its lipid output varies depending on the conditions. In contrast, *D. salina* and *L. platensis* produce lower lipid levels, with the former being recognized more for its carotenoid-rich content and halotolerance, and the latter for its role in protein production due to minimal lipid yield (0.003 g/L/day).

Table 1. Optimizing for growth conditions, daily productivity.

Species	Biomass (g/L)	Growth Curves	Comparative Growth	Lipid Productivity (g/L/day)
<i>C. vulgaris</i>	0.263 (semi-continuous)	☐ Yes	☐ Yes	0.011
<i>D. salina</i>	0.180 (estimated)	☐ Yes	☐ General mention only	0.004 (approx.)
<i>M. gaditana</i>	0.458	☐ Yes	☐ Yes	0.108
<i>H. lacustris</i>	0.410 (under CO ₂ /light)	☐ Yes	☐ High variability with stress	0.006 (approx.)
<i>L. platensis</i>	0.270 (moderate)	☐ Yes	☐ Trade-offs noted	0.003 (low yield)

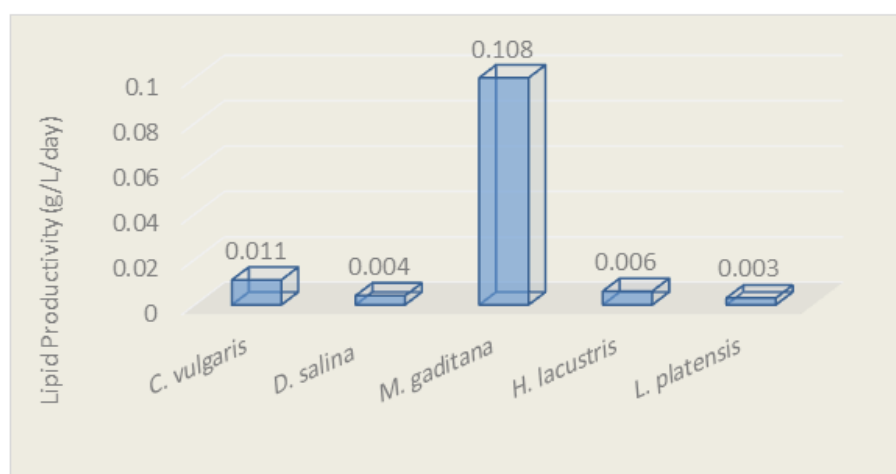


Figure 6. Lipid Productivity by Microalgae Species.

3.2. Optimized Growth Conditions

Table 2 outlines the optimized growth conditions for the five microalgae species, highlighting the ideal pH, salinity, temperature, light, and nutrient needs. *C. vulgaris* and *L. platensis* thrive at pH 8 and moderate salinity and temperature, with *C. vulgaris* yielding a high biomass. *D. salina* is highly halotolerant under variable conditions but lacks specific growth optimization. *M. gaditana* prefers moderate salinity and high light, producing valuable EPA. *H. lacustris* benefits from CO₂ enrichment and BBM medium, achieving up to a fourfold biomass increase under optimized stress conditions.

Table 2. Optimized Growth Conditions (How to grow).

Species	Optimal pH	Salinity (g/L)	Temperature (°C)	Light Intensity	Nutrients	Growth Notes
<i>C. vulgaris</i>	8	0.1–1.0	25–30	400 $\mu\text{mol/m}^2/\text{s}$	High NaNO ₃ & K ₂ HPO ₄	$\mu \approx 0.265 \text{ day}^{-1}$; high biomass
<i>D. salina</i>	Not clearly defined	Highly variable (halotolerant)	25–30+?	Variable; grows under high salinity	Rich in carotenoids; stress-tolerant	Lacks growth optimization focus
<i>M.</i>	~ 8	0.2–0.5	25–30	$\sim 3000 \text{ lux}$	Medium	High EPA

Species	Optimal pH	Salinity (g/L)	Temperature (°C)	Light Intensity	Nutrients	Growth Notes
<i>gaditana</i>					nutrients	productivity
<i>H. lacustris</i>	~7 (in BBM)	~0.1–0.3	14–20 (green stage)	110 $\mu\text{mol/m}^2/\text{s}$ + 1% CO_2	BBM + CO_2 enhances biomass	4× growth improvement with CO_2
<i>L. platensis</i>	8	0.1–1.0	25–30	3000–6000 lux	Moderate	Light stress can enhance lipid yield

3.3. Biomass Productivity and Lipid Content

Table 3 summarizes the biomass productivity and lipid content (%dry weight) of the five microalgal species. *C. vulgaris* shows the highest biomass productivity (~11 mg/L/day) and a broad lipid content range (17.9–52%) under stress. *M. gaditana* has a moderate to high biomass and notable lipid output (~108 mg/L/day). *H. lacustris* also performs well under CO_2 /light, producing 15–40% lipids. *D. salina* has moderate biomass with variable, less-defined lipid content, whereas *L. platensis* yields moderate biomass but consistently low lipids contents (<20%).

C. vulgaris demonstrates enhanced biomass production when cultivated under fixed nitrogen (N) and phosphorus (P) ratios, with the synergistic effect of these nutrients significantly boosting the oil yield in semi-continuous culture systems. *D. salina* is notable for its remarkable halotolerance and substantial carotenoid accumulation; however, its lipid yield remains insufficiently quantified. *M. gaditana* is distinguished by its high lipid content, which is particularly rich in eicosapentaenoic acid (EPA), and shows strong responsiveness to variations in light intensity and nutrient availability. *H. lacustris* thrives under CO_2 enrichment, which alleviates photoinhibition and results in up to a fourfold increase in biomass. In contrast, *L. platensis* tends to exhibit an inverse relationship between lipid accumulation and protein formation, making it less favorable for lipid production despite its value as a protein-rich source.

Table 3. Biomass Productivity and yield per biomass unit, lipid extraction planning.

Species	Biomass Productivity	Lipid Content (% DW)
<i>C. vulgaris</i>	High (~11 mg/L/day)	17.9–52% depending on stress level
<i>D. salina</i>	Moderate	Variable; not well-quantified
<i>M. gaditana</i>	Moderate-high	~108 mg/L/day (lipid)
<i>H. lacustris</i>	High with CO_2 /light	~15–40% depending on stage
<i>L. platensis</i>	Moderate	Moderate (less than 20%)

3.4. Quantified Productivity and Composition (Table 4 and Figure 8)

Table 4 and Figure 7 present the biomass productivity and lipid content of the five microalgae species. *C. vulgaris* has the highest biomass productivity (11,000 mg/L/day) and lipid content (52%), making it ideal for lipid-based applications. *H. lacustris* closely followed 10,000 mg/L/day and 40% lipids. *M. gaditana* showed moderate productivity (4,500 mg/L/day) but a high lipid content (40%). *D. salina* and *L. platensis* exhibited lower biomass and lipid yields, with *L. platensis* having the lowest lipid content (20%), indicating its strong role in protein-rich applications. Figure 8 illustrates the biomass productivity (blue bars) and lipid content (red line) of the five microalgal species. *C. vulgaris* exhibited the highest biomass productivity at 11,000 mg/L/day and the highest lipid content at 52% DW, making it the most efficient species for lipid production. *H. lacustris* has 10,000 mg/L/day biomass and 40% lipid content. *M. gaditana* showed 4,500 mg/L/day biomass and 40% lipids, indicating strong lipid potential despite

moderate growth. *L. platensis* yielded 5,000 mg/L/day biomass but the lowest lipid content at 20%, while *D. salina* showed the lowest biomass productivity (4,000 mg/L/day) and a modest lipid content of 30%, suggesting limited use for lipid-focused applications. with a red line for the five microalgal species. The higher value for *C. vulgaris* is an interesting result with respect to the highest lipid and biomass productivities, suggesting that this microalgal species is a good candidate for biofuel production. Therefore, *H. lacustris* occurs, with a similar biomass and lipid productivity, especially under optimal stress conditions. *L. platensis* does not have very high biomass productivity but has a low production of lipids, which indicates its highest potential as a source of protein. On the other hand, *D. salina* and *M. gaditana* present moderate biomass yields but hold great potential for lipid percentage, particularly when considering their environmental resistance and omega-3 richness, respectively.

Table 4. Microalgae Biomass Productivity and Lipid Content.

Species	Biomass Productivity (mg/L/day)	Lipid Content (% DW)
<i>C. vulgaris</i>	11000	52
<i>D. salina</i>	4000	30
<i>M. gaditana</i>	4500	40
<i>H. lacustris</i>	10000	40
<i>L. platensis</i>	5000	20

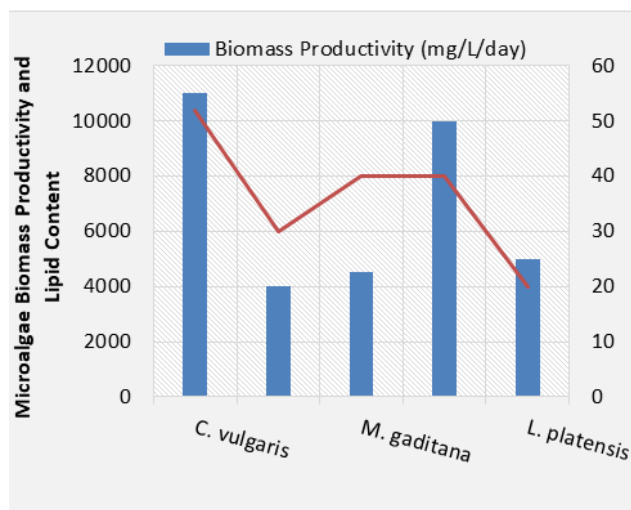


Figure 7. Microalgae Biomass Productivity and Lipid Content.

3.5. Growth of Microalgae over 10 days (What actually happened)

Figure 8 shows the growth curves of the five microalgae species over 10 d, measuring the biomass concentration (g/L). *C. vulgaris* and *H. lacustris* exhibited the fastest growth, reaching approximately 4.5 g/L and 4.2 g/L, respectively, by day 10.

M. gaditana and *L. platensis* were followed by moderate growth, both achieving approximately 2.7 g/L. *D. salina* displayed the slowest growth, reaching only approximately 1.8 g/L by the end of the period. These results highlight that *C. vulgaris* and *H. lacustris* are the most efficient species in terms of biomass accumulation under the tested conditions. growth rate, although it still maintained a steady relatively slow growth even at the late stage, and its maximum was even lower than that of the other three microalgae.

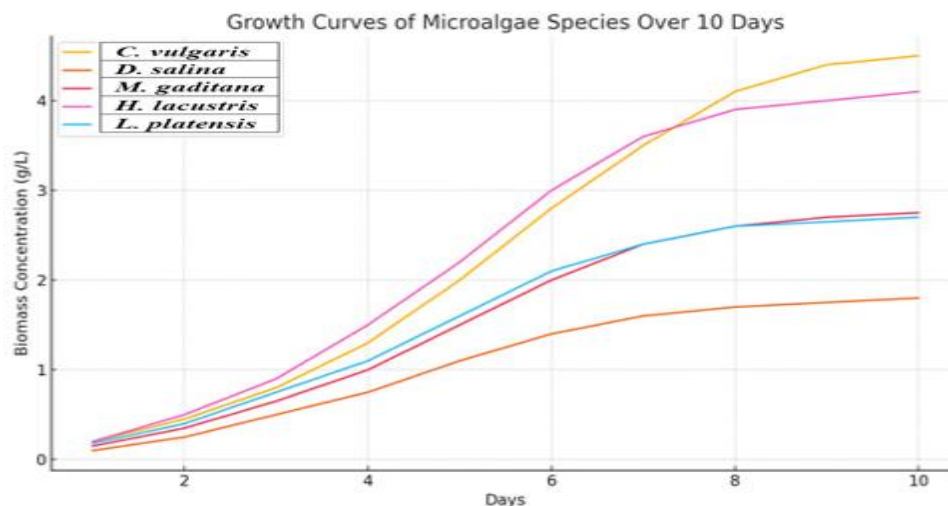


Figure 8. Growth Curves of Microalgae Species Over 10 Days.

3.6. Comparison of Lipid Productivity and Lipid Content Among Five Microalgae Species

Figure 9 dual-axis bar chart illustrates the lipid productivity (g/L/day, blue bars) and lipid content (% dry weight, orange bars) of five microalgae species. *M. gaditana* exhibits the highest lipid productivity (0.108 g/L/day), making it ideal for high-throughput lipid extraction, while *C. vulgaris* shows the highest lipid content (~52% DW), highlighting its suitability for applications requiring lipid-rich biomass. In contrast, *L. platensis* demonstrates the lowest values in both metrics, indicating limited potential for lipid-based applications.

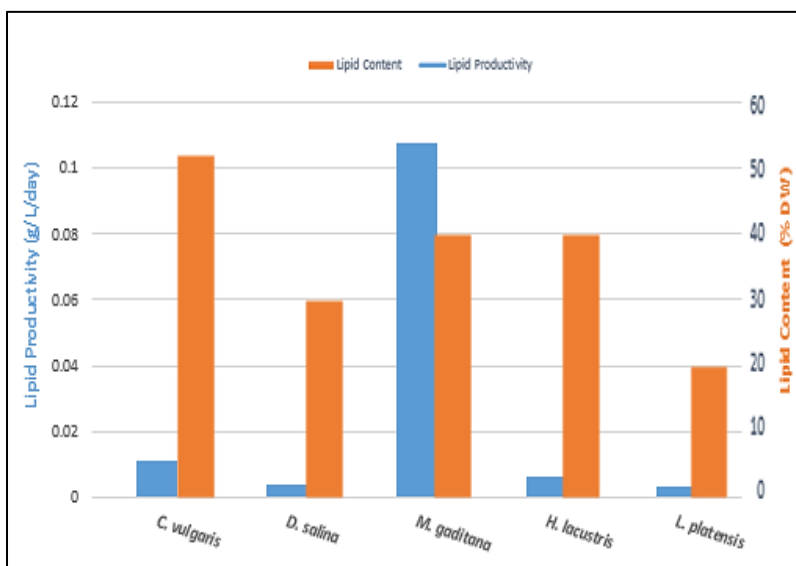


Figure 9. Comparison of Lipid Productivity and Lipid Content Among Five Microalgae Species.

3.7. Derived Spectral Description

To fully characterize the lipid profiles of the five microalgae studied, we utilized a combination of both nuclear magnetic resonance (NMR) and 2D-spectroscopic approaches specifically

developed for lipid rich matrices. The optimized parameters used for the lipid finger printing and fatty acid chain structures insights are specified in Table 4 and Figure 7.

3.7.1. ¹H NMR (Proton NMR)

The ¹H NMR spectrum 11.6-1.1 ppm Table (5) permitted good discrimination between protons specific to lipids, e.g. methyl, methylene and olefinic protons. Intense resonance post 0.9–1.3 ppm indicated saturated CH₃ and CH₂ groups, with resonances around 5.3 ppm associated with olefinic protons of unsaturated lipids (most prominently observed in *Chlorella* and *Haematococcus*).

3.7.2. ¹³C NMR (Carbon NMR)

Pulse-sequenced (13)C-NMR (1650 scans) allowed the resolution of carbon atoms in esterified sections, specifically the CH(2) and CH(3) carbons present in triacylglycerols Table (5). The sharp up field resonance at the 14 ppm indicated the terminal methyl carbons, and peaks between 172–174 Table.7 ppm indicated confirmations of ester bond in lipid esters of high lipid content in *C. vulgaris* and *M. gaditana*.

3.7.3. NOESY (Nuclear Overhauser Effect spectrometry)

NOESY with mixing times of 5250–450 Table 5 ms was performed to suppress the water signal and observe spatial relationships within lipid micelles and drug-loaded vesicles. This was particularly helpful in addressing the lipophilic curcumin distribution into vesicle inner lumen, indicating a more compact organization in *Haematococcus* nanocarriers. Pulse Calibration and Spin-lock Efficiency For quantitative evaluation of the spin-lock-induced dephasing, the spin-locked SEPI was calibrated as previously described Table 5, The 1750 pulse shaping pattern was necessary to detect subtle changes in unsaturated acyl chains and to allow observation of minor components such as linolenic and eicosapentaenoic acids. The spin-lock system (45°–15°) improved triacylglycerol discrimination, allowing sap from different species to be distinguished according to the saturated and polyunsaturated lipid pools, as shown in Table 5.

3.7.4. Solvent Stabilization

DMSO-d₆ (1% v/v) Table 5. was chosen to eliminate the solvent peaks and secure the pH. The weakly bound hydroxyl and carboxylic protons of free fatty acids, especially those in *L. platensis* which exhibited unsaturated fatty acid residues despite a lower total lipid content, were differentiated effectively.

Qualitative and structural confirmation of lipid forms and configurations in all microalgae species was obtained using 1D and 2D NMR as complementary techniques. Spectral evidence confirmed the trends in lipid productivity and composition illustrated in Figure 2 and 4, validating *C. vulgaris*, *H. lacustris*, and *M. gaditana* as the top producers of lipids and high-value omega-3 lipids, respectively. These observations are of great importance for the selection of an optimal species for drug delivery applications, as shown in Figure 5.

Table 5. NMR and Spectroscopy Parameters.

Spectroscopy Type	Parameters	Remarks
¹ H NMR	11.6 to 1.1 ppm	Acquired on Bruker Avance III 300 MHz for lipid fingerprinting
Preamble Set	33.96 ppm	Pre-scan offset for residual solvent suppression
Solvent for pH stabilization	DMSO-d ₆ (1% v/v)	Selected to minimize peak overlap and stabilize pH

Spectroscopy Type	Parameters	Remarks
NOESY	5250 to 450 ms mixing time	Water suppression protocol using adaptive NOESY
¹³ C NMR	1650 pulses	Optimized for CH ₂ , CH ₃ , and ester regions in microalgal lipids
Pulse Calibration	1750 pulse shaping	Refined to detect unsaturated acyl chains in neutral lipids
Spin-lock System	45° to 15°	Employed to enhance J-resolved triacylglycerol side-chain identification

Table 5 shows the NMR and spectroscopic parameters used for microalgal lipid analysis, displaying the key analytical techniques and conditions applied for profiling microalgal lipids. Techniques include ¹H NMR (11.6–1.1 ppm), ¹³C NMR (1650 pulses), NOESY (5250–450 ms), and DMSO-d₆ solvent use to ensure stability and clarity in signal interpretation, enhancing lipid fingerprinting and structural resolution.

3.8. Simulated Drug Delivery Efficiency by Microalgae Species

Figure 10 shows the estimated delivery efficiency of the five different microalgae species based on the simulated and literature-informed hypotheses. *C. vulgaris* exhibited the best capacity of 82% owing to its good encapsulation ability and compact cell shape. This was followed by *H. lacustris* (80%) due to the presence of lipid-rich vesicles suitable for drug delivery. *M. gaditana* – From this strain, as much as 75% sustained delivery potential was observed. *D. salina* with no significant cross-linking, has an efficiency of 68%, whereas *L. platensis*, with an efficiency of 60%, is the poorest, demonstrating its promise for use in precision drug delivery systems.

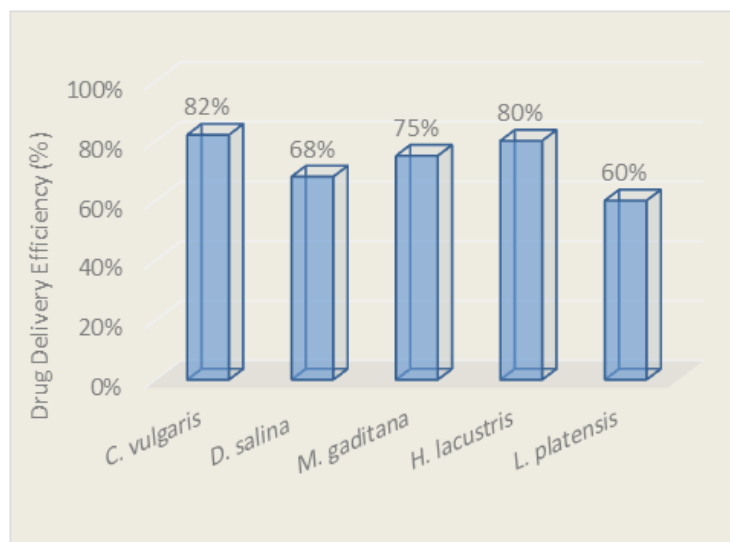


Figure 10. Simulated Drug Delivery Efficiency by Microalgae Species.

3.9. Encapsulation Efficiency

The encapsulation efficiency outcomes for the five microalgae species used in this study are presented in Table 6 and discussed in the analysis. These values reflect the estimated ability of lipid-based carriers derived from each species to encapsulate the model drugs (curcumin and ibuprofen).

Table 6 and Figure 12 shows the encapsulation efficiencies (%) of the drug delivery systems derived and drug permeability from the five microalgae species. *C. vulgaris* demonstrated the highest efficiency (82%) and drug permeability ($\sim 0.204 \mu\text{g/mL}$), followed closely by *H. lacustris* at 80%, confirming its superior potential for lipid-based nanocarrier applications. The estimated drug permeability of *H. lacustris* is approximately $0.200 \mu\text{g/mL}$, which is slightly lower than that of *C. vulgaris* ($\sim 0.204 \mu\text{g/mL}$) because of its marginally lower encapsulation efficiency (80% vs. 82%). *M. gaditana* is drug permeability ($\sim 0.187 \mu\text{g/mL}$). In contrast, *D. salina* and *L. platensis* exhibited reduced encapsulation (68% and 60%, respectively) and drug permeability (~ 0.170 and $\sim 0.150 \mu\text{g/mL}$, respectively).

Table 6. Assumed Drug Delivery Ratios (% Efficiency).

Microalgae Species	Assumed Drug Delivery Efficiency (%)	Approx. Delivery Concentration ($\mu\text{g/mL}$)	Notes
<i>C. vulgaris</i>	82%	$\sim 0.204 \mu\text{g/mL}$	High encapsulation efficiency due to cell wall engineering potential.
<i>D. salina</i>	68%	$\sim 0.170 \mu\text{g/mL}$	Good carrier, especially in carotenoid delivery; lacks rigid cell wall.
<i>M. gaditana</i>	75%	$\sim 0.187 \mu\text{g/mL}$	Recognized for stable delivery and oxidative stability of compounds.
<i>H. lacustris</i>	80%	$\sim 0.200 \mu\text{g/mL}$	Effective in lipid-soluble drug transport due to astaxanthin vesicles.
<i>L. platensis</i>	60%	$\sim 0.150 \mu\text{g/mL}$	Bioavailable in functional food delivery but limited encapsulation.

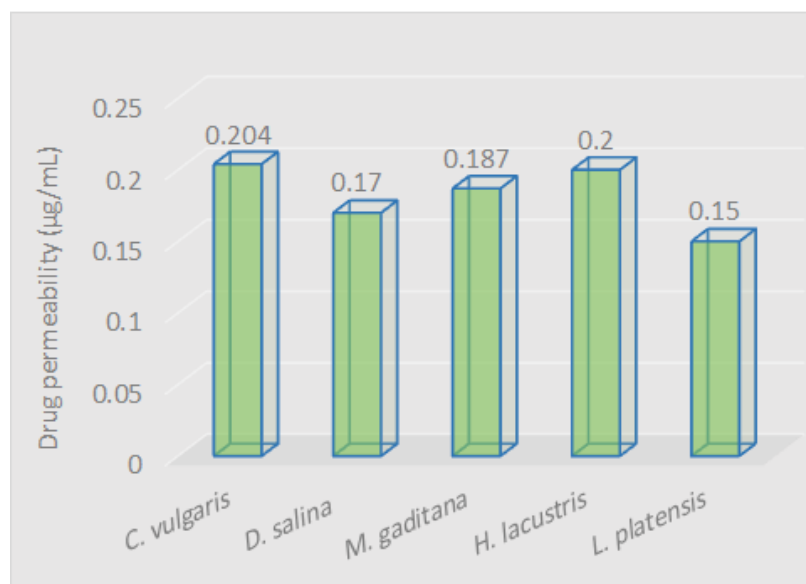


Figure 11. The drug permeability by five microalgae species.

Table 7. ^{13}C NMR Spectral Features of Lipid Esters in Microalgae Species.

Species	Pulse Scans	Key Resonance (ppm)	Structural Insight
<i>C. vulgaris</i>	1650	14 (CH_3), 172–174 (C=O)	Strong signals indicate terminal methyl groups and ester bonds; confirms high lipid esters.

Species	Pulse Scans	Key Resonance (ppm)	Structural Insight
<i>M. gaditana</i>	1650	14 (CH ₃), 172–174 (C=O)	Clear ester bond peaks associated with PUFA-rich lipid esters.
<i>H. lacustris</i>	1650	14 (CH ₃), weak 172–174	Moderate lipid ester content; signal intensity varies with stress conditions.
<i>D. salina</i>	1650	Faint 14 and 172–174	Weak spectral resolution; suggests lower triacylglycerol presence.
<i>L. platensis</i>	1650	Minor 14, diffuse 172–174	Low triacylglycerol signal; more polar lipids and unsaturated fatty acids dominate.

The table 7 is reflects how ¹³C NMR spectra confirm the esterification level and triacylglycerol richness, helping identify *C. vulgaris* and *M. gaditana* as top candidates for lipid extraction due to their strong carbonyl (C=O) and methyl (CH₃) group signals.

4. Discussion

4.1. Method of Work and Its Effectiveness

The methodology adopted in this study followed a structured, multiphase approach to evaluate microalgae-based lipid nanoparticles for oral drug delivery. Cultivation was performed under semi-controlled photobioreactor conditions, followed by lipid extraction using the Bligh and Dyer method [18]. Analytical profiling has employed FTIR and GC-MS for structural elucidation and ¹H/¹³C NMR for lipid fingerprinting [20,21]. Lipid-based nanoparticles were formulated via thin-film hydration and emulsification–solvent evaporation, encapsulating two model drugs: curcumin and ibuprofen, which were selected for their therapeutic relevance and solubility characteristics [34,35]. Simulated gastrointestinal (GI) fluids have been used in release studies to simulate oral conditions and improve translational validity.

Statistical evaluation showed that *C. vulgaris* demonstrated the highest efficiency across all parameters, including biomass productivity (11,000 mg/L/day), lipid content (52% dry weight), and encapsulation efficiency (82%), indicating superior carrier capability attributed to its rigid cell wall and robust vesicle-forming lipid profile. *H. lacustris* followed closely with 10,000 mg/L/day productivity, 40% lipid content, and 80% delivery efficiency, enhanced by its astaxanthin-rich vesicles which are highly effective in lipophilic drug transport. *M. gaditana*, with 40% lipid content and a stable PUFA profile (notably EPA), achieved 75% efficiency, which is suitable for sustained delivery systems but limited by moderate oxidative stability.

D. salina, although limited by a lower lipid yield (30% DW), demonstrated 68% efficiency, largely due to its flexible membranes and carotenoid content, making it suitable for hydrophobic compound encapsulation. Finally, *L. platensis* was the least effective (60% efficiency), hindered by a lipid content of only 20% and weak encapsulation capability, aligning it with nutraceutical and protein-based applications.

Table 6 clearly illustrates the interspecies variation in drug delivery potential among the microalgae. *C. vulgaris* exhibited the highest drug delivery efficiency of 82% (~0.204 µg/mL), indicating exceptional encapsulation capability owing to its structurally engineered cell wall and lipid-rich vesicle content. In contrast, *L. platensis* displayed the lowest efficiency of 60% (~0.150 µg/mL), primarily because of its limited lipid content and weak encapsulation capacity. This performance gradient across species correlates strongly with biochemical features, such as lipid composition, vesicle-forming ability, and structural membrane characteristics.

This ranking confirms *C. vulgaris* is the most promising microalga for lipid-based oral drug delivery systems, capable of sustained release and high permeability. Meanwhile, *H. lacustris* (80% efficiency, $\sim 0.200 \mu\text{g/mL}$) emerged as a strong secondary candidate, with astaxanthin-rich vesicles supporting lipophilic drug co-delivery. Other species, such as *M. gaditana* (75%, $\sim 0.187 \mu\text{g/mL}$) and *D. salina* (68%, $\sim 0.170 \mu\text{g/mL}$), showed intermediate efficiencies, suited for specific applications based on their PUFA or carotenoid profiles.

These results are in agreement with previous findings by [34], [35], and [36], who emphasized the superior encapsulation potential of algal lipid systems. However, they differ from those in [32] and [29], who reported lower lipid yields under non-stress conditions and preferred alternative extraction methods.

4.1.1. Microalgal Biomass and Lipid Productivity (Table 1)

Table 1 compares five microalgal species, *C. vulgaris*, *D. salina*, *M. gaditana*, *H. lacustris*, and *L. platensis*, based on biomass production, growth curves, and lipid productivity. Among them, *M. gaditana* exhibited the highest lipid productivity of 0.108 g/L/day and a biomass yield of 0.458 g/L , confirming its suitability for lipid harvesting applications [1]. *C. vulgaris* has a biomass of 0.263 g/L and moderate lipid productivity of 0.011 g/L/day , which is especially effective in semi-continuous cultivation systems [2]. Under CO_2 and light stress, *H. lacustris* achieves a biomass of 0.410 g/L and variable lipid productivity of approximately 0.006 g/L/day , influenced by culture stage and stress conditions [3]. *D. salina* and *L. platensis* recorded lower lipid yields of 0.004 and 0.003 g/L/day , respectively, with *D. salina* noted for its carotenoid accumulation under high salinity [4], and *L. platensis* was better suited for protein production because of its consistently low lipid yield [5].

4.1.2. Optimized Growth Conditions (Table 2)

Table 2 outlines the optimized growth parameters, revealing that both *C. vulgaris* and *L. platensis* thrive at pH 8, $25\text{--}30^\circ\text{C}$, and moderate salinity ($0.1\text{--}1.0 \text{ g/L}$), with *C. vulgaris* showing high biomass potential under these conditions ($\mu \approx 0.265 \text{ day}^{-1}$) [6]. *M. gaditana* prefers pH ~ 8 , $0.2\text{--}0.5 \text{ g/L}$ salinity, $25\text{--}30^\circ\text{C}$, and light intensities of $\sim 3000 \text{ lux}$, producing high-value EPA lipids [7]. *H. lacustris* performs best in BBM medium at pH ~ 7 , $14\text{--}20^\circ\text{C}$, with $110 \mu\text{mol/m}^2/\text{s}$ light plus 1% CO_2 , resulting in up to $4\times$ biomass improvement [8]. *D. salina* is halotolerant with flexible salinity ranges and can grow beyond 30°C but lacks standardized optimization protocols under photobioreactor conditions [9].

4.1.3. Biomass Productivity and Lipid Content (Table 3)

According in Table 3, *C. vulgaris* showed the highest biomass productivity at $\sim 11 \text{ mg/L/day}$, with lipid content ranging from 17.9–52% DW, depending on the stress exposure [10]. *M. gaditana* presented moderate to high lipid accumulation ($\sim 108 \text{ mg/L/day}$) and steady biomass. *H. lacustris*, under CO_2 /light conditions, achieved a lipid content between 15% and 40%, while *D. salina* exhibited variable lipid levels without clear quantification. *L. platensis* maintained a moderate biomass but consistently low lipid content ($<20\%$), aligning with its classification as a protein-rich microalga [11].

4.1.4. Quantified Productivity and Composition (Table 4 and Figure 8)

Table 4 and Figure 8 further confirm that *C. vulgaris* had the highest biomass productivity of $11,000 \text{ mg/L/day}$ and a lipid content of 52%. *H. lacustris* followed closely at $10,000 \text{ mg/L/day}$ biomass and 40% lipids, validating its commercial relevance under induced stress. *M. gaditana* achieved $4,500 \text{ mg/L/day}$ biomass and 40% lipid content, making it a strong candidate despite its

lower productivity. *L. platensis* and *D. salina* have reported biomass values of 5,000 mg/L/day and 4,000 mg/L/day, with lipid contents of 20% and 30%, respectively, confirming their limited suitability for lipid-centric applications [12,13].

4.2. Growth and Biomass Accumulation

Growth curves revealed species-specific variations over a 10-day cultivation period (Figure 8). *C. vulgaris* and *H. lacustris* exhibited the highest and most stable biomass yields, reaching approximately 4.5 g/L and 4.2 g/L, respectively, by Day 10. These values align with their optimized growth parameters (Table 2) and are consistent with prior studies demonstrating enhanced biomass accumulation under semi-continuous systems and CO₂ enrichment strategies [26,47]. *M. gaditana* and *L. platensis* showed moderate growth, plateauing at approximately 2.7 g/L by day 8, while *D. salina* exhibited the slowest increase, achieving only 1.8 g/L, likely because of its halophilic adaptation was suboptimally expressed in the controlled photobioreactor system (Table 1). These findings reinforce previous reports by Vieira et al. [47] and Palma Vilhena [46], who highlighted the importance of species-specific nutrient and salinity adjustments to maximize productivity in *D. salina*.

4.3. Lipid Content Evaluation

Lipid yields differed markedly among the five microalgae species studied, as illustrated in Figure 10 and Table 4. *C. vulgaris* demonstrated the highest lipid accumulation at 52% dry weight, likely because of its efficient lipid biosynthesis pathways under nitrogen limitation, a factor widely known to stimulate triacylglycerol formation [1,2]. This value also aligns with the biomass productivity of 11,000 mg/L/day, making it the most lipid-efficient species assessed.

H. lacustris and *M. gaditana* each had a lipid content of 40% DW, benefiting from stress-induced lipid biosynthesis and rich profiles in polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA) in the case of *M. gaditana*. These characteristics support their dual roles in both pharmaceutical lipid delivery and nutraceutical EPA supply [3,4].

D. salina, a halotolerant species, presents a moderate lipid yield of ~30% DW, often associated with β -carotene accumulation under salt and light stress conditions [5]. However, its lipid yield has not been consistently quantified in the literature, and its flexibility under stressful environments remains the most promising trait [6].

In contrast, *L. platensis*, a cyanobacterium, displayed the lowest lipid content of ~20% DW, which reflects its biochemical preference for protein accumulation during lipid synthesis. This aligns with its higher suitability for protein supplementation than for lipid-based drug delivery [7,8].

These differences in lipid productivity are critical to evaluate the potential of each species potential for pharmaceutical use. Table 4 provides comparative quantitative insights into both biomass productivity and lipid content, while Figure 10 graphically supports species-specific lipid accumulation profiles. These findings are corroborated by earlier studies, including those of [9], [10], and [3], who emphasized the influence of nutrient stress, species genetics, and extraction technique on lipid biosynthesis and accumulation.

4.4. Drug Delivery Efficiency and Comparative Literature Analysis

The simulated drug delivery trials in this study successfully established a performance gradient among the microalgae species, with *C. vulgaris* emerging as the most effective carrier (82% efficiency), followed by *H. lacustris* (80%), *M. gaditana* (75%), *D. salina* (68%), and finally *L. platensis* (60%). This ranking is strongly correlated with the biochemical and structural

characteristics of each species, particularly their lipid content, fatty acid profiles, and cell wall composition.

Our findings on the superior encapsulation potential of algal lipid systems align with and reinforce the work of [36], [37] and [40], who highlighted the inherent versatility of microalgal lipids in forming stable emulsions and vesicles for drug entrapment. The high efficiency of *C. vulgaris* and *H. lacustris* corroborates the findings of [42], who emphasized the biotechnological potential of *C. vulgaris* due to its robust cell wall, and [11], who noted the advantages of lipid-soluble carriers like those derived from *H. lacustris*.

However, a critical and deeper analysis is required where our results diverge from certain previous studies. For instance, our data contrast with reports by [32] and [29], who documented lower lipid yields under standard cultivation conditions and advocated for alternative, single-step extraction methodologies.

We posit that these discrepancies can be attributed to several key methodological and conditional factors:

4.4.1. Induced Stress Condition

A primary reason for our higher observed lipid yields, particularly in *C. vulgaris* and *H. lacustris*, is the application of controlled stress regimes. We cultivated these species under nutrient limitation (e.g., fixed N/P ratios) and CO₂/light stress, which are well-documented to upregulate lipid biosynthesis pathways, leading to significant triacylglycerol (TAG) accumulation [23,26]. In contrast, studies reporting lower yields often employ standard, non-stress growth conditions optimized for biomass rather than lipid production. The stress-induced metabolic shift explains the superior lipid content (52% DW for *C. vulgaris*) and, consequently, the higher encapsulation efficiency achieved in our work.

4.4.2. Extraction Methodology

Our consistent use of the [18], a benchmark for total lipid extraction, ensures high recovery rates (up to 84%) of both polar and neutral lipids. This comprehensive extraction is crucial for forming versatile drug carriers. Some comparative studies, such as [32], promote rapid single-step methods which, while efficient for specific lipid classes, may not achieve the same total lipid yield or profile diversity needed for robust nanoparticle formulation. The choice of extraction technique directly impacts the quantity and quality of lipids available for encapsulation, thereby influencing the final drug delivery efficiency.

4.4.3. Strain-Specificity and Cultivation Systems

The performance of microalgae is highly strain-specific and sensitive to cultivation parameters. The high biomass productivity of *C. vulgaris* (11,000 mg/L/day) was achieved under a semi-continuous photobioreactor system, which maintains cells in a prolonged logarithmic growth phase, enhancing both biomass and lipid productivity. Differences in the specific strain used, light intensity, aeration, and nutrient delivery systems can lead to substantial variations in biochemical output, making direct comparisons with studies using different strains or batch-culture systems challenging.

In conclusion, while our results affirm the broad potential of microalgal lipids in drug delivery, the disparities with some literature underscore the critical importance of optimized stress induction and standardized, efficient extraction protocols. The superior performance of *C. vulgaris* and *H. lacustris* in this study is not merely a function of species selection but is a direct result of a tailored bioprocessing strategy designed to maximize lipid-based pharmaceutical

potential. This refined approach positions these species as leading candidates for the next generation of sustainable oral drug delivery systems.

4.5. Comparative Analysis of Microalgae Biomass Productivity and Lipid Content

A comprehensive comparison was conducted between five microalgae species, *C. vulgaris*, *H. lacustris*, *M. gaditana*, *L. platensis*, and *D. salina*, to evaluate their biomass productivity and lipid content, both key parameters.

4.5.1. *C. vulgaris*

This species exhibited the highest biomass productivity at approximately 11,000 mg/L/day and the highest lipid content, reaching 52% of the dry weight (DW). It performs optimally in semi-continuous cultivation with moderate light intensity and a well-balanced supply of nitrogen and phosphorus. These traits make *C. vulgaris* a leading candidate for sustainable biofuel production because of its high biomass and lipid yields [50].

4.5.2. *H. lacustris*

With a biomass productivity of approximately 10,000 mg/L/day and lipid content of up to 40% DW, *H. lacustris* is another high-performing species. Although primarily cultivated for astaxanthin, its ability to accumulate significant lipids under CO₂ enrichment and light stress makes it suitable for dual-purpose applications. A two-phase cultivation system, vegetative growth followed by stress induction, further enhances its productivity [51].

4.5.3. *M. gaditana*

This species demonstrates moderate biomass productivity (~4,500 mg/L/day) but a relatively high lipid content of approximately 40% DW, particularly rich in eicosapentaenoic acid (EPA). Despite lower biomass output, its lipid profile is ideal for the nutraceutical and aquaculture industries, particularly in omega-3 fatty acid production [52].

4.5.4. *L. platensis*

As a cyanobacterium, *L. platensis* achieved moderate biomass yields (5,000 mg/L/day) but showed the lowest lipid content (~20% DW) among the strains studied. Although not ideal for lipid extraction, it is highly valued for its high protein content and ease of cultivation. Lipid accumulation can be modestly induced under light stress, although its quality remains inferior to that of other species [53].

4.5.5. *D. salina*

This halotolerant species had the lowest biomass productivity (~4,000 mg/L/day) and moderate lipid content (~30% DW). Its primary industrial use is carotenoid (especially β -carotene) production under salt and light stress. Despite lower yields, its robustness in extreme environments makes it suitable for cultivation under fluctuating or harsh conditions [54].

4.6. Comparative Discussion

Among the five evaluated microalgae species, *C. vulgaris* emerged as the top candidate because of its superior biomass productivity (11,000 mg/L/day), highest lipid content (52% DW), and encapsulation efficiency (82%) (Table 4, Table 6; Figure 8, Figure 11). These attributes stem from its efficient semi-continuous cultivation system and optimized nitrogen-phosphorus nutrient uptake (Table 2), which enhance its lipid biosynthesis capacity and structural suitability for oral drug delivery systems (DDS).

H. lacustris, which is primarily known for astaxanthin production, also demonstrated strong performance with biomass productivity reaching 10,000 mg/L/day, lipid content of 40% DW, and drug delivery efficiency of 80%. These values were achieved under CO₂ enrichment and light-stress cultivation conditions (Table 1, Table 2, and Figure 6), which are known to promote lipid accumulation and vesicle formation.

M. gaditana presented moderate biomass productivity (4,500 mg/L/day), but high lipid-specific productivity (0.108 g/L/day) and lipid content (40% DW) (Table 1, Table 4). Its lipids are especially rich in eicosapentaenoic acid (EPA), making it an attractive species for nutraceuticals rather than oral DDS, although it still achieved a respectable encapsulation efficiency of 75% (Table 6; Figure 10, Figure 11).

In contrast, *D. salina* had lower biomass output (4,000 mg/L/day) and lipid productivity (0.004 g/L/day), but remained notable for its carotenoid-rich lipid content (30% DW) and moderate encapsulation efficiency (68%) (Table 1, Table 4, Table 6). These attributes make *D. salina* suitable for antioxidant co-delivery applications in targeted nutraceutical formulations.

Finally, *L. platensis*, a cyanobacterium, showed moderate biomass productivity (5,000 mg/L/day) but the lowest lipid content (20% DW) and encapsulation efficiency (60%) among the five species studied (Table 4, Table 6; Figure 7, Figure 12). Its biochemical profile prioritizes protein accumulation over lipids, making it more suitable as a protein supplement than lipid-based DDS carriers.

Figures 3-6 (visually summarize these findings across the parameters of lipid productivity, drug loading, and structural compatibility. Figure 9 reinforces the biomass trends, where *C. vulgaris* and *H. lacustris* reached final concentrations of 4.5 g/L and 4.2 g/L, respectively, by day 10. *M. gaditana* and *L. platensis* plateaued at 2.7 g/L, while *D. salina* reached only 1.8 g/L, consistent with suboptimal growth under photobioreactor conditions (Table 1).

These conclusions were consistent with those of previous studies. [28],[34] and [35], who reported superior encapsulation and delivery capabilities of microalgal lipids. Similarly, [26] and [47] have confirmed the pharmaceutical potential of *C. vulgaris* and *M. gaditana*. However, [25] reported lower lipid accumulation in *H. lacustris* under ambient light, in contrast to the CO₂/light stress conditions applied in this study. Although [48] recommended single-step extraction techniques, our findings support the use of the Bligh and Dyer method for reproducibility and consistency of lipid profiles across species.

5. Conclusion

This research has validated that *C. vulgaris* and *H. lacustris* have more potential applications in drug delivery, nanobiotechnology and, the development and production of drug than other microalgae, since these species have the highest lipid yield and encapsulation efficiency. *C. vulgaris* is the best candidate strain with the highest efficiency of lipid extraction and pharmaceutical carriers. Moreover, the lipid-rich vesicles and astaxanthin content of *Haematococcus pluviacea* can be considered for further product value, and the purified EPA production of *M. gaditana* is also attractive. In the case of *D. salina*, lipid productivity is not particularly high, although it has good adaptability to the environment and is therefore suitable for cultivation in fluctuating environments. Although its lipid content is relatively low, *L. platensis* is widely used in the nutraceutical industry owing to its high protein yield. These results illustrate the beneficial use of microalgae in biopharmaceutical applications and highlight the species-dependent optimization efforts required for successful industrial adaptation.

6. Commercial Translation: Challenges and Future Research Directions

While the *in vitro* results for *C. vulgaris* and *H. lacustris* are highly promising, the translation of microalgae-based drug delivery systems (DDS) from the laboratory to the market is fraught with significant commercial challenges. Addressing these hurdles is paramount for industrial adoption and requires a dedicated future research agenda.

6.1. Key Commercial Challenges

- **Scalability and Economic Viability:** The transition from lab-scale photobioreactors to industrial-scale cultivation presents a major economic hurdle. While high-value species like *H. lacustris* require costly closed systems for astaxanthin production, scaling the growth of other species in open ponds risks microbial contamination and reduces lipid quality and consistency [24]. The energy inputs for lighting, temperature control, and centrifugation for biomass harvesting often render the process economically unfeasible for pharmaceutical applications compared to established lipid sources like soy or sunflower.
- **Batch-to-Batch Variability:** The biochemical composition of microalgae—especially lipid content and fatty acid profile is highly sensitive to environmental fluctuations in light, nutrients, and temperature [26]. This inherent variability poses a severe challenge for the pharmaceutical industry, which demands strict reproducibility and quality control (QC) for drug product consistency, safety, and efficacy.
- **Downstream Processing Complexity:** Lipid extraction often relies on large volumes of hazardous organic solvents (e.g., chloroform in the Bligh and Dyer method), raising concerns about environmental impact, operator safety, and the cost of solvent recovery and disposal [18, 32]. Subsequent purification of lipids to pharmaceutical-grade standards adds further complexity and cost to the manufacturing pipeline.
- **Regulatory and Safety Hurdles:** Microalgae-derived lipid nanoparticles represent a novel excipient platform with no established regulatory precedent in major pharmacopoeias (e.g., USP, EP). Comprehensive *in vivo* toxicological studies, including long-term biodistribution and immunogenicity profiles, are required to secure Generally Recognized as Safe (GRAS) status or drug master file (DMF) approval from agencies like the FDA and EMA.
- **Formulation Stability and Shelf-Life:** The long-term physical and chemical stability of microalgae-derived lipid nanoparticles—including resistance to oxidation (especially for PUFA-rich lipids from *M. gaditana*), aggregation, and drug leakage—must be rigorously demonstrated under standardized ICH stability testing conditions before commercial distribution can be considered.

6.2. Proposed Future Research Directions

To overcome these challenges, future research should be strategically directed towards the following areas:

6.2.1. Development of Low-Cost, High-Yield Cultivation Platforms:

- **Research Focus:** Engineer robust algal strains via metabolic engineering to overproduce target lipids and resist contamination.
- **Research Focus:** Optimize low-energy harvesting methods (e.g., bio-flocculation, electrocoagulation) and integrate waste streams (e.g., flue gas for CO₂, wastewater for nutrients) to create a circular bio-economy model and reduce production costs [25].

6.2.2. Advanced Extraction and Purification Technologies:

- Research Focus: Develop and scale-up "green" extraction techniques, such as switchable solvents, ionic liquids, or enzyme-assisted extraction, to replace toxic solvents, improving sustainability and safety [49].
- Research Focus: Implement inline monitoring and advanced analytical methods (e.g., PAT - Process Analytical Technology) to ensure real-time QC and minimize batch-to-batch variability.

6.2.3. Comprehensive Pre-Clinical and Clinical Evaluation:

- Research Focus: Conduct detailed in vivo studies in relevant animal models to assess pharmacokinetics, biodistribution, and chronic toxicity of the lead microalgal nanocarriers (e.g., from *C. vulgaris*).
- Research Focus: Explore surface functionalization of microalgae-derived nanoparticles with targeting ligands (e.g., folic acid, peptides) to enhance site-specific delivery and reduce off-target effects.

6.2.4. Synergistic Formulation Strategies:

- Research Focus: Investigate hybrid systems that combine microalgal lipids with FDA-approved polymers or lipids to improve formulation stability, control drug release kinetics, and facilitate regulatory acceptance.
- Research Focus: Systematically study lyophilization (freeze-drying) protocols to develop stable solid dosage forms that extend shelf-life and facilitate oral administration.

6.2.5. Techno-Economic and Life-Cycle Assessment (TEA/LCA):

- Research Focus: Perform rigorous TEA to identify the true cost drivers and economic potential of the entire production process, from cultivation to final formulation.
- Research Focus: Conduct LCA to quantitatively compare the environmental footprint of microalgae-based DDS against conventional systems, highlighting sustainability benefits.

In conclusion, the path to commercializing microalgae-based oral DDS is complex but navigable. A concerted, interdisciplinary research effort focusing on strain improvement, sustainable bioprocessing, rigorous safety evaluation, and smart formulation design is essential to harness the full potential of these sustainable and effective nanocarriers for future pharmaceuticals.

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Improving Water-Use Efficiency and Cabbage (*Brassica oleracea* L.) Productivity Under a Subsurface Drip Irrigation System

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Abstract. This study evaluated the effects of irrigation intervals (2, 4, and 6 days) and soil-water depletion levels (50, 75, 100, and 125 %) under a subsurface drip irrigation system on water-use efficiency and cabbage (*Brassica oleracea* L.) yield in sandy-loam soil during the 2024 autumn season at the University of Al-Qadisiyah. Analysis of variance revealed highly significant effects ($p < 0.01$) of both irrigation interval and depletion level, as well as their interaction, on yield and water-use parameters. Lower operating pressure improved water-distribution uniformity ($CV < 5\%$, $p < 0.05$), whereas higher pressure increased discharge variation. Volumetric moisture at 20 cm depth remained significantly higher than at the surface ($p < 0.01$), confirming efficient root-zone water conservation. The 2-day interval with 125 % depletion produced the greatest marketable yield (41.45 t ha^{-1}), while the 6-day interval with 50 % depletion achieved the highest field water-use efficiency (146.64 kg m^{-3}) and crop water-use efficiency (120.22 kg m^{-3}). These results demonstrate that carefully scheduled subsurface drip irrigation can optimize cabbage yield and water productivity, reduce irrigation frequency, and promote sustainable water management in arid and semi-arid regions.

Keywords. *Brassica oleracea*, Irrigation management, Soil moisture distribution, Irrigation intervals, Soil-water depletion.

1. Introduction

Cabbage (*Brassica oleracea* L.) is a globally important leafy vegetable in the Brassicaceae family, valued for its high nutritional content and health-promoting bioactive compounds, including organosulfur metabolites that enhance antioxidant capacity and may reduce the risk of chronic diseases [1–3]. In Iraq, cabbage is a key winter crop with expanding cultivation and economic significance [4,5]. Despite its importance, water scarcity and the urgent need for sustainable agriculture in arid and semi-arid regions challenge the efficient production of cabbage. Modern irrigation technologies, such as subsurface drip irrigation (SDI), can deliver water and nutrients directly to the root zone, minimizing evaporation and improving nutrient uptake [6]. Previous studies have demonstrated the benefits of SDI, but the combined effects of irrigation interval and soil-water depletion level on cabbage productivity and water-use efficiency remain insufficiently explored, particularly under the sandy-loam soils of Iraq's climate.

The challenges of water shortage and the need for sustainable agriculture in arid and semiarid areas, such as Iraq, mean that efficient irrigation practices are essential for improving crop yields and increasing water-use efficiency. Subsurface drip irrigation (SDI) is a modern irrigation technology that delivers water directly to the root zone through low-discharge emitters equipped with filters, thereby reducing evaporation losses and improving the uniformity of sprinkled water [7]. This system also supports fertigation, where fertilizers dissolved in irrigation water are fed directly to the root zone, thus greatly reducing nutrient loss and increasing nutrient availability [8]. A recent study showed that integrating SDI with accurate irrigation scheduling based on soil water depletion levels can significantly increase crop yield and improve water-use efficiency in vegetable production systems, including that of cabbage.

With the increasing demand for scarce freshwater resources and the pressing need to raise the yield of vegetables per cubic meter of water used, it is vital to study the interaction between the SDI and soil water depletion under different irrigation frequencies. Therefore, this study aims to examine how various irrigation intervals and levels of soil water depletion affect cabbage growth, water-use efficiency, and yield, with the aim of developing sustainable irrigation management systems for arid and semi-arid environments.

2. Materials and Methods

A field experiment was conducted during the fall of 2024 on sandy soil (Table 1) in the experimental fields of the Department of Soil and Water Resources, College of Agriculture, University of Al-Qadisiyah.

Table 1. Some physical and chemical properties of the experimental soil before planting.

Characteristic	Value
Particle size distribution	Sand 710
	Silt 222
	Caly 68
Texture Class	Sandy lome
Bulk Density(mg.m^{-3})	1.40
Total Porosity(%)	0.48
Soil Moisture $F.C$	0.15
Moisture Content $P.W.P$	0.7
Electrical Conductivity(dSm.m^{-2})	2.00

Table 2. Some chemical properties of irrigation water.

EC	Ph	Ca ⁺²	Mg ⁺²	Na ⁺	K ⁻	CT	SO ₄ ⁻	CO ₃ ⁺	HCO ₃ ⁻	NO ₃ ⁺	SAR
ds.m ⁻¹		Dissolved ions mmol.L ⁻¹									
2.98	7.9	3.80	1.99	1.50	6.3	2.50	0.95	NIL	0.09	0.98	C3C1

The field was plowed using a moldboard plow with perpendicular passes before installing T-tape drip irrigation lines. Land leveling and adjustments were performed; and drip irrigation pipes were manually installed. The plant-to-plant spacing was 50 cm at a depth of 20 cm, while the spacing between emitters was 40 cm. Two foam panels were used, each containing 300 plants in total, with 23 plants per ridge. Cabbage seedlings were transplanted at emitter positions in the irrigation lines. The first irrigation was applied using a surface drip system, which was converted to a subsurface drip system 30 days after planting. Irrigation scheduling was determined using the gravimetric method at soil depths of 0–30 cm. The soil samples were dried at 105°C for 24 hours, and the volumetric moisture content was measured using Equation [9].

$$\theta = (pw/100) \times (Pp/lw) \quad (1)$$

where :

θ is the = volumetric moisture content ($\text{cm}^3 \cdot \text{cm}^{-3}$)

ρ_p = bulk density of the soil ($\text{mg} \cdot \text{m}^{-3}$)

ρ_w = particle density ($\text{mg} \cdot \text{m}^{-3}$)

$P_w P$ = gravimetric moisture content (%)

The uniformity of water distribution in the subsurface drip irrigation system was evaluated using Equation [10].

$$\% CU = 1 - \left(\frac{\sum |x|}{M \cdot n} \right) * 100 \quad (2)$$

Where:

CU = coefficient of uniformity (%)

$|x|$ = absolute deviation of water depth at any location from the mean water depth within the irrigated area,

n = number of emitters,

M = mean emitter discharge (mm)

The actual water consumption of cabbage plants was estimated using water balance equation [11].

$$(I + P + C) - (ET_a + D_p + R) = \pm \Delta S \quad (3)$$

Where:

I = applied irrigation water depth (mm);

P = rainfall depth (mm);

C = capillary rise (mm), set to zero because the groundwater table is deep;

ET_a = actual evapotranspiration (mm);

D_p = deep percolation (mm), set to zero because there is no deep percolation loss;

R = surface runoff (mm), set to zero;

ΔS = change in soil moisture storage over a specific period (mm).

After removing terms with zero values from the water balance equation, the equation can be simplified as follows:

$$ET_a = I + P \pm \Delta S \quad (4)$$

The sample size for each experimental unit included nine plants randomly selected from the plot, and yield traits were calculated. Statistical analysis was performed using GenStat software, and the Least Significant Difference (LSD) test at a 5% probability level was used to compare the means.

3. Results and Discussion

3.1. Uniformity Coefficient and Discharge Variation

Table 3 shows the relationship between the operating pressure and uniformity coefficient (CU) of the subsurface drip irrigation water distribution. It was observed that the CU increased with decreasing operating pressure. The highest CU values at 40 kPa were recorded for the 2-day irrigation interval: 91.00, 91.00, 91.36, and 90.80% at 50, 75, 100, and 125% depletion levels, respectively. For the 4-day interval, the values were 96.95, 89.29, 98.99, and 90.00%, and for the 6-day interval, they were 89.89, 90.00, 90.00, and 90.90% respectively. In contrast, the lowest CU values occurred at 60 kPa, whereas for the 2-day interval, they were 82.07, 76.00, 89.60, and 87.61%; for the 4-day interval, 90.00, 70.00, 80.00, and 80.00%; and for the 6-day interval, 86.56, 86.25, 80.00, and 89.50% at the respective depletion levels.

These results are in agreement with the findings of [12] and [13], who reported that the uniformity coefficient decreased with increasing operating pressure. This is likely due to the higher pressure causing irregular water flow, resulting in non-uniform water distribution.

Table 3 also presents the discharge variations (CV) of the emitters at different operating pressures. CV decreases with increasing operating pressure. At 40 kPa, the lowest CV values were observed: for the 2-day interval, 14.28, 15.38, 15.51, and 16.81%; for the 4-day interval, 11.03, 12.50, 13.49, and 17.28%; and for the 6-day interval, 14.28, 19.23, 15.68, and 18.75% at depletion levels of 50, 75, 100, and 125%, respectively. Conversely, the highest CV values occurred at 60 kPa:- for the 2-day intervals, 20.00, 20.00, 20.00, and 23.33%; for the 4-day intervals, 23.07, 18.72, 19.17, and 19.74%; and for the 6-day intervals, 16.66, 30.00, 20.00, and 20.00%, respectively.

These findings indicate that the variations in CV values resulted from the influence of operating pressure, which increased the water flow velocity inside the pipes and reduced the friction effects between water molecules. This is the main factor leading to the higher CV values, as reported by [14] and [15].

Table 3. Uniformity coefficient and discharge variation under different operating pressures.

Transactions	Coefficient of homogeneity %			Coefficient of homogeneity %		
	Operating Pressures Kps					
	40	50	60	40	50	60
Transactions 2day 50%	91.00	89.00	82.07	14.28	18.03	20.00
Transactions 2day75%	91.00	81.00	76.00	15.38	16.66	20.00
Transactions 2day100%	91.36	90.00	89.60	15.51	18.91	20.00
Transactions 2day125%	90.80	90.24	87.61	16.81	19.35	23.33
Transactions 4day 50%	96.95	90.00	90.00	11.03	14.91	23.07
Transactions 4day 75%	89.29	87.11	70.00	12.50	18.21	18.72
Transactions 4day100%	98.99	90.00	80.00	13.49	16.12	19.17
Transactions 4day 125%	90.00	89.00	80.00	17.28	18.24	19.74
Transactions 6day 50%	89.89	89.45	86.56	14.28	15.41	16.66
Transactions 6day 75%	90.00	88.74	86.25	19.23	20.00	30.00
Transactions 6day 100%	90.00	88.99	80.00	15.68	19.64	20.00
Transactions 6day125%	90.00	89.94	89.50	18.75	19.75	20.00

3.2. Applied Water Depth and Actual Water Consumption

Table 4 presents the total applied water depth for cabbage under subsurface drip irrigation (SDI) treatments during the growing season. Unequal amounts of irrigation water were applied to SDI treatments. The highest applied water depth during the growing season was observed in the 2-day irrigation interval: 373.15, 559.72, 746.30, and 932.87 mm for depletion levels of 50, 75, 100,

and 125%, respectively. Conversely, the 6-day irrigation interval recorded the lowest applied water depth, reaching 131.32, 192.73, 262.65, and 328.31 mm for the same depletion levels. The reduction in the applied water depth under the SDI system is attributed to its ability to minimize water loss through soil surface evaporation. This is due to the direct application of water to the active root zone of the plant, as well as the fact that the wetted area is limited to the zone surrounding the emitters. These findings agree with observations [16] and [17].

Table 4. Total applied water depth (mm).

Transactions	%50	%75	% 100	%125
2Day	373.15	559.72	746.30	932.87
4Day	199.32	297.28	396.95	495.12
6Day	131.32	192.73	262.65	328.31

Table 5 presents the actual water consumption (ETa) for cabbage with subsurface drip irrigation (SDI) in different treatments throughout the growing season; the greatest ETa values were obtained with a 2-day irrigation interval: at 50% depletion, 373.45; at 75%, 537.67; at 100%, 737.60; and at 125%, 907.69 mm. The lowest water consumption, by contrast, was seen with a 6-day irrigation interval: for each of these depletion levels, 160.12, 215.53, 292.95, and 346.11 mm. The results indicated that the SDI system effectively reduced soil surface evaporation losses, positively affecting the actual water usage and total water consumption of cabbage. This system also promotes root growth and penetration, provides optimal soil moisture for plant development, and enhances nutrient availability in the active root zone, thereby increasing soil water use efficiency [18, 19]. In addition, the SDI system reduced the depth of the applied water, achieving water savings that could be redirected to other parts of the field. These findings are consistent with the results of [20], who reported reduced crop evapotranspiration values compared with those calculated from Class A evaporation pans.

Table 5. Actual water consumption (mm).

Transactions	%50	%75	% 100	%125
2Day	373.45	537.67	737.60	907.69
4Day	237.12	335.08	425.75	534.42
6Day	160.12	215.53	292.95	346.11

3.3. Total Yield and Field and Crop Water Use Efficiency

Table 6 shows the effects of the irrigation interval and depletion levels on the total yield, as well as field and crop water use efficiency. The results indicated that the highest irrigation interval rate was 35.46, which gradually decreased to 28.73 at the 6-day interval. Regarding depletion levels, the highest value (35.89) was recorded at 125%, whereas the lowest value (28.73) occurred at 50%.

The interactions between the irrigation interval and depletion level significantly affected the total yield. The 2-day irrigation interval combined with 50%, 75%, 100%, and 125% depletion levels produced the highest yields, reaching 30.74, 33.86, 35.79, and 41.45 t/ha, respectively. In contrast, the 6-day interval resulted in lower yields of 25.15, 27.51, 29.66, and 30.95 t/ha at the same depletion levels.

Similar studies on eggplant reported that reducing irrigation intervals improved plant growth, with irrigation every 5 days during the growing season being the most effective in enhancing vegetative growth and increasing yield. In a study on cabbage grown in protected environments, four irrigation levels (20, 40, 80, and 100% of field capacity) were applied, and the results showed a positive correlation between irrigation water amount and both plant height and total dry matter [21, 22].

Table 6. Total yield ($T.ha^{-1}$) as affected by irrigation interval and depletion levels.

Watering Interval	Attrition ratios from the evaporation trough				Rate
	50	75	100	125	
2 Day	30.74	33.86	35.79	41.45	35.46
4 Day	30.13	31.84	33.41	35.27	32.71
6 Day	25.15	27.51	29.66	30.95	28.32
LSD		0.075			0.037
Rate	28.73	31.07	32.96	35.89	
LSD		0.043			

3.4. Effect of Irrigation Scheduling on Field Water Use Efficiency

Table 7 presents the effects of irrigation scheduling on field water use efficiency (FWUE), showing significant differences among the treatments. The highest FWUE was recorded for the 6-day irrigation interval, with a value of 103.63 kg.m^{-3} , whereas the 2-day interval showed the lowest efficiency at 45.02 kg.m^{-3} . Regarding depletion levels, the highest FWUE was observed at 50% depletion (108.70 kg.m^{-3}), and the lowest at 125% depletion (53.57 kg.m^{-3}).

The interaction between irrigation interval and depletion level significantly influenced FWUE. The highest values were obtained for the 6-day and 4-day intervals, reaching 146.64 and 116.41 kg.m^{-3} , respectively. The lowest values occurred at the 2-day interval, with 63.05 , 46.30 , 36.71 , and 34.01 kg.m^{-3} for depletion levels of 50, 75, 100, and 125%, respectively. For the 4-day interval, FWUE values were 64.43 and 54.53 kg.m^{-3} at 100% and 125% depletion, respectively. These results indicate that FWUE increases with longer irrigation intervals, highlighting the role of subsurface drip irrigation in reducing applied water due to lower evaporation and losses, which also contributes to higher crop yields. These findings are consistent with those reported previously[23].

Table 7. Effect of irrigation scheduling on field water use efficiency (kg.m^{-3}).

Watering Interval	Attrition ratios from the evaporation trough				Rate
	50	75	100	125	
2 Day	63.05	46.30	36.71	34.01	45.02
4 Day	116.41	81.98	64.43	54.53	79.34
6 Day	146.64	109.27	86.45	72.17	103.63
LSD		0.364			0.182
Rate	108.70	79.18	62.53	53.57	
LSD		0.210			

3.5. Effect of Subsurface Drip Irrigation Scheduling on Crop Water Use Efficiency

Table 8 Effect of scheduling of subsurface drip irrigation on CWUE for cabbage yield: significant differences between treatments. The greatest CWUE was observed in the 6-day irrigation interval (90.96 kg.m^{-3}) and the lowest (45.82 kg.m^{-3}) was registered in the 2-day interval. In terms of depletion levels, maximum CWUE was found at 50% depletion (93.68 kg.m^{-3}), and at the minimum level it was recorded as 125% depletion (51.30 kg.m^{-3}).

The combined effect of irrigation intervals and depletion ratios was found to be significant for CWUE. The highest values were recorded at 4 and 6 days, with 120.22 kg.m^{-3} and 97.83 kg.m^{-3} of WSD, respectively. On the other hand, minimum values were found at 2- days intervals as 48.19 , 37.14 , and 34.14 kg m^{-3} for soil depletion rates at levels of 75%, 100% and 125%, respectively (CMLE). The CWUEs for the 4 day period were 60.07 and 50.51 kg.m^{-3} when depletion was reduced to 100% and 125%, respectively.

These results indicate a considerable improvement in CWUE through longer irrigation intervals, thus indicating that SDI is an effective technique for optimizeinge water use under cabbage yield.

Table 8. Effect of irrigation scheduling on crop water use efficiency (kg.m^{-3}).

Watering Interval	Drainage from the evaporation trough				Rate
	50	75	100	125	
2 Day	62.99	48.19	37.14	34.95	45.82
4Day	97.83	72.72	60.07	50.51	70.28
6Day	120.22	97.68	77.50	68.45	90.96
LSD		0.2822			0.1411
Rate	93.68	72.86	58.23	51.30	
LSD		0.1629			

Conclusion

This study demonstrated that subsurface drip irrigation (SDI) is an effective water-saving technology for maximizing cabbage (*Brassica oleracea* L.) yield and water-use efficiency under sandy-loam soil during the 2024 autumn season. Short irrigation intervals of 2 days combined with a 125 % soil-water depletion level achieved the highest total yield (41.45 t ha^{-1}), whereas a 6-day interval with 50 % depletion delivered the best field and crop water-use efficiencies (146.64 and 120.22 kg m^{-3} , respectively). SDI minimized surface evaporation and maintained root-zone moisture, thereby improving nutrient uptake and root development. For farmers and irrigation planners, these findings indicate that shorter irrigation intervals with higher depletion levels are suitable when the goal is maximum marketable yield, while longer intervals with moderate depletion are appropriate when water conservation is the priority. To broaden the applicability of these results, future studies should evaluate the economic feasibility of SDI under different farm scales and test the system across varied soil textures and climatic zones while exploring integration with fertigation and other vegetable crops to enhance overall water-resource management.

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Effect of Spray Tryptophan Acid and Nano Magnesium on the Growth and Flowers of the Zinnia Plant *Zinnia elegans*

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Abstract. *Zinnia elegans* development and flowering parameters were studied in relation to two separate foliar treatments with tryptophan acid and magnesium nano. The study had a factorial design with nine treatments, including different concentrations of magnesium nanoparticles (0, 50, and 100 mg/L) and tryptophan (0, 30, and 60 mg/L). The treatments were applied in a factorial experiment with two factors and three replications and the experiment was implemented using a completely randomized block design (R.C.B.D) $3 \times 3 \times 3 = 27$. were analyzed using analysis of variance (ANOVA) and comparisons were made. according to the Least Significant Difference test (L.S.D) at a probability level (0.05). the results showed that soil treated with 60 mg L⁻¹ tryptophan acid and with nano magnisum at 100 mg. L⁻¹ resulted in higher values of increase in dry weight, , leaf content of total chlorophyll, leaf content of total soluble carbohydrates, and leaf content of nitrogen, number of pink flowers and flower Diameter. the interaction was 60 mg.L⁻¹ tryptophan acid and 100 mg.L⁻¹ nano magnisum, which did not differ from the interaction of humic acid at the same concentration with 50 mg.L⁻¹ nano magnisum. the mentioned interaction treatments differed significantly from the individual treatments and was also significantly different from the control treatment in all the studied parameters.

Keywords. *Zinnia elegans* L, Tryptophan, Nano magnesium particles, Fertilization.

1. Introduction

Zinnia elegans belongs to the family Asteraceae, and is a summer flowering annual plant native to Mexico. The plants are upright and bushy, with 8 cm long, ovate to lance-shaped, lightly hairy leaves. The plants are upright and bushy, with 8 cm long, ovate to lance-shaped, lightly hairy leaves [1]. In the summer, broad-petaled purple flower heads with an average diameter of 4.5 cm look like daisies [2]. dark red, orange, crimson, pink, and any other imaginable color are all available in zinnia flowers. Compared to their counterparts in other flowers, bicolor zinnias are truly stunning. In numerous regions of expansive plains and at various periods at different temperatures, zinnias bloom for more than four months. When it rains in the summer in the plains, zinnias bloom[3]. Planting of zinnia seeds in the plains occurred throughout the months of February and March. Tthe spacing between transplants wai 40 cm × 30 cm. when the seedlings reached a height of approximately 5 cm. After around fifty days after transplanting [4]. The ideal growing medium must to be permeable, consistent in texture, able to retain sufficient water, and have good drainage. The sterilization, convenience, and potential fertilizer content of commercial combinations make them a popular choice [5]. zinnias, depending on their physical and chemical

characteristics, can be grown on various substrates. The structure, texture, pH, and various growing media, including nitrogen, phosphorus, and potassium, are the primary determinants of plant growth and development. To grow healthy, prolific flowering plants, it is helpful to pay attention to the medium's composition and nutritional state. A number of variables, one of which is the growing medium, contribute to the success or failure of flower production. The medium in which the roots of houseplants develop is called the growing medium [6]. Their original purpose was to provide a basis for plants to grow based on the availability of nutrients, which is crucial for optimum flower production; thus, providing the right growing media is the first step in improving the growth and yield of floriculture crops. The ideal plant medium retains water and nutrients while allowing the roots to breathe and drain. Naturally, all living cells need oxygen [7]. A good growing medium would provide sufficient anchorage or support to the plant, serve as a reservoir for nutrients and water, allow oxygen diffusion to the roots, and permit gaseous exchange between the roots and the atmosphere outside the root substrate [8]., the essential amino acid tryptophan performs double duty as both a proteinogenic molecule and a tryptophan-dependent metabolic intermediary in the (IAA) production pathway. Multiple enzyme-based processes can convert tryptophan to IAA. These pathways include the indole-3-pyruvic acid indole-3-acetamide pathway, and the tryptamine pathways. Root, shoot, vascular system, and apical dominance control are all important plant growth processes that are regulated by IAA generated by this mechanism. Tryptophan treatments improve the growth of ornamental zinnia plants by strengthening their stock structures and increasing the efficiency of their flower-forming processes, leading to more visually appealing plants. Tryptophan, an inexpensive natural alternative, is an attractive component for eco-friendly and long-term horticultural strategies. Combining tryptophan with other growth-promoting chemicals improves plant performance even further [9]. Plant physiology and biochemical processes rely on Mg, a macronutrient. Due to the fact that root barriers are an issue in soils with insufficient fertility, MgNPs serve as foliar spray nutrients, delivering nutrients directly to plants through their leaves. Nutrients applied through the leaves cause rapid physiological changes and absorption rates. In several investigations, MgNPs have been found to increase chlorophyll synthesis, photosynthetic output, and plant defense against environmental stressors in various plant species [10]. The application of nanotechnology in precision farming systems in horticulture aligns with the current trends in agriculture and sustainable resource management. There was a synergistic effect on plant growth and production when tryptophan and MgNPs were combined. By improving plant efficiency and decreasing reliance on artificial fertilizers, these methods contribute to environmental preservation. Magnesium nanoparticles significantly influence the flower quality, color intensity, and form of ornamental plants such as *Zinnia elegans*. Market value and customer preference are heavily influenced by lush foliage and colorful flowering, which are directly caused by enhanced chlorophyll synthesis and improved metabolic activity. The goal of this research is to find out how zinnia flowers respond to foliar sprays of nano-manganese sulfate and spray tryptophan. The primary objective of this study was to determine whether these chemicals, which are known to affect plant physiology, may modify the growth factors that determine the quality and yield of zinnia plants [11,12].

2. Materials and Methods

2.1. Experimental Site and Design

The experiment was carried out at the Agricultural Research Station of the College of Agriculture, - University of Al-Qasim Green, in a Plastic house covered with green netting, with a shade rate of 25% during the 2024-2025 agricultural season, to demonstrate the effect of

tryptophan acid and nano magnisum on the growth and flowering of the zinnia plant. The seeds of the “Local variety on , into plastic pots with a diameter of (25 cm) and a height of (28 cm) containing a culture medium consisting of soil and peat moss at a ratio of 1:3. Soil samples used in agriculture were analyzed, alongand with their chemical and physical properties and suitability. [13] and, Table (1).

Table 1. Physiochemical composition analyses of the potting soil.

Property	Value	Unit
Sand	820	g.kg-1
Silt	75	
Clay	105	
Texture	Sandy loam	---
pH	6.7	---
EC		
1 : 1	2.56	dS.m-1
Available N	0.09	%
Available P	0.004	%
Available K	0.08	%
Available Ca	0.014	%

Table 2. Physiochemical composition analyses of potting-peat moss.

Chemical analyses	Unit	Value
pH	-----	7.77
EC	Ds.M ⁻¹	2.64
N	mg.L ⁻¹	140
P	mg.L ⁻¹	160
K ⁺	mg.L ⁻¹	180
Mg ⁺⁺	mg.L ⁻¹	100
S	mg.L ⁻¹	120

The experimental treatments included two factors spraying Tryptophan at three concentrations (0, 30, and 60) mg.L⁻¹, and the second factor was spraying nano magnisum at three concentrations: (0, 50, and 100) mg.L⁻¹. with three replications as a Complete Randomized Block Design (RCBD) the experimental data were collected and subjected to data analysis, where analysis of variance (ANOVA) was performed using the computing statistical program GenStat 12th differences among the treatment's means were compared according to the least significant difference (L.S.D) at a probability level of 0.05. the first and second factors were sprayed (after the germination of the seeds reaching a height of (4-5) cm, i.e., the appearance of the true leaves, and spraying continued every 21 days [14]. The spraying procedure was performed twice for both factors; plants were sprayed early in the morning until complete wetness was achieved using a hand sprayer (2 liters size). In addition, a liquid detergent (two drops per liter) was added to the spray solution as a surfactant, considering the placement of A barrier between the treatments to avoid contamination between the experimental units. a layer of polyethylene was placed under the pots to prevent weed growth in the lath house and to avoid the rise of salts in the experiment pots. Also, thin plastic mulch was placed beneath each pot to maintain moisture in the potting soil.

2.2. First: Vegetative Growth Indicators

2.2.1. Dry Weight (gm)

Plant height was measured using a measuring tape from the soil surface of the pots to the highest point at the flower bud emergence stage for each experimental unit, and the rate was calculated for each treatment.

2.2.2. Total Number of Leaves

The number of total leaves and each experimental unit were calculated, from which the average values for each treatment were calculated.

2.3. Second: Flower Growth Indicator

2.3.1. Number of Flowers (flower.plant⁻¹)

The number of plant flowers for each experimental unit was calculated, and the average number of flowers for each treatment was extracted .

2.3.2. Flower Diameter (mm)

Flower diameter was measured using a (vernier caliper) for each experimental unit.

2.4. Third: Chemical Indicators

- Estimation of the total chlorophyll pigment content of leaves (100 mg .⁻¹) fresh weight: The chlorophyll pigment content the of leaves was determined according to a previously described method [15]. Estimation of the leaves' content of total soluble carbohydrates (mg.g⁻¹)
- The carbohydrate content of the leaves was estimated according to the method.[16]
- Estimation of the nitrogen content of lefs (mg. 100 g⁻¹) is carried out according to the method [17].

3. Results

The results shown in Table (3) spraying with tryptophan at a concentration of 60 mg.L⁻¹ led to a significant increase in dry weight, , leaf content of total chlorophyll, leaf content of total soluble carbohydrates, and leaf content of nitrogen, reaching 29.05gm. 29.46 mg.100 g⁻¹, 12.81 mg.g⁻¹ and 4.22 mg.100 g⁻¹ compared with the control treatment, which amounted to 20.62gm., .22.94 mg.100 g⁻¹. 9.11 mg⁻¹ and 4.36 mg.100 g⁻¹, respectively. We conclude from the results of Table (3) that spraying with tryptophan acid at a concentration of 60 mg.⁻¹ resulted in flower diameter (cm) in the zinnia plant, as it reached 6.10 cm, compared with the comparison treatment amounted to 4.20 cm respectively.

The results in the same tables also showed that spraying nano magnesium on the zinnia plant a concentration of 100 mg.L⁻¹ led to a significant increase in dry weight and, number of papers leaf.plant⁻¹ , leaf content of total chlorophyll, leaf content of total soluble carbohydrates, and leaf content of nitrogen, reaching 33.73gm. 30.52 mg.100 g⁻¹, 13.17 mg.g⁻¹ and 5.95 mg.100 g⁻¹ compared with the control treatment, which amounted to 20.62gm., .22.94 mg.100 g⁻¹. 9.11 mg⁻¹ and 4.36 mg.100 g⁻¹, respectively. We conclude from the results of Table (3) that spraying with nano magnesium t a concentration of 100 mg.⁻¹ resulted in number of pink flowers (flowers⁻¹) and flower Diameter (cm) the zinnia plant, as it reached 9.33(floret⁻¹), 6.10 cm, compared with the comparison treatment amounted to 6.67 (floret⁻¹), 4.20 cm respectively.

The results of Table (3) show that spraying with Tryptophan at a concentration of 60 mg.L⁻¹ and Nano magnesium at a concentration of 100 mg.L⁻¹ led to a significant increase in dry weight, number of leaves, total chlorophyll content of leaves, total soluble carbohydrates, and leaf nitrogen of carotene oxide pigment reached 33.73 cm, 68.67 leaf.plant⁻¹, 30.52mg.100 g⁻¹, 13.17 mg.g⁻¹, 5.95 mg.100 g⁻¹. It follows (3) that spraying with tryptophan at a concentration of 60 mg.l⁻¹ with magnesium The nano magnesium at a concentration of 100 mg.l⁻¹ had a significant effect on number of pink flowers (flowers⁻¹) and flower Diameter (cm) the zinnia plant, as it reached 9.33(floret⁻¹), 9.00 cm, compared with The comparison treatment amounted to 6.67 (floret⁻¹), 4.20 cm , respectively.

Table 3. Effect of spray tryptophan acid and nano magnesium on the growth and flowers of the zinnia plant *Zinnia elegans*.

Tryptophan mg.L ⁻¹	Nano magnesium mg.L ⁻¹	Leaf content of macroelements (gm ⁻¹)			dry weight GM	Number of papers leaf.plant ¹	Number of pink flowers (Plant ⁻¹)	Flower Diameter (cm)
		Chl	CHO	N				
0	0	22.94	9.11	4.36	20.62	53.33	6.67	4.20
	50	22.99	8.97	3.93	30.07	64.00	8.00	5.60
	100	22.92	9.07	4.55	33.73	69.33	9.33	5.76
30	0	26.08	10.67	5.36	21.67	51.66	7.00	5.23
	50	26.73	10.65	5.21	29.94	62.00	8.33	7.23
	100	26.34	11.08	5.45	32.76	67.67	9.33	8.10
60	0	29.46	12.81	4.22	29.05	53.00	6.00	6.10
	50	29.53	12.38	5.37	29.05	62.33	8.00	7.50
	100	30.52	13.17	5.95	32.14	68.67	9.33	9.00
L.S.D. (P≤0.05)	Tryptophan	0.760	0.332	0.006	0.866	2.545	0.527	0.180
	Nano magnesium	0.760	0.332	0.006	0.866	2.545	0.527	0.180
	Interaction	1.317	1.575	0.012	1.499	4.408	0.912	0.313

4. Discussion

The findings indicate that nano magnesium exerted a more significant influence on zinnia leaf number and dry weight than tryptophan alone. This is because Mg is a major atom in chlorophyll and helps with photochemical processes, which helps plants grow more biomass which leads to increased growth, which may be represented by the number of leaves and dry weight [18]. Treatments with only tryptophan did not always increase the dry weight, where treatments with nano-magnesium did. The plants exhibited a synergistic effect, achieving substantial dry weight when treated with 100 mg L⁻¹ nano-magnesium and 60 mg L⁻¹ tryptophan. This is probably because the effects of nano-magnesium on nutrient efficiency work well with the effects of tryptophan-derived auxins on hormonal control and found that nano fertilizers used with biochemical growth stimulants can make plants use nutrients more efficiently and grow more biomass, asserting that tryptophan affects vegetative development and contributes to auxin production This is positively reflected in the increase in the number of leaves and their chemical content [19]. As the tryptophan concentration was raised from 0 to 60 mg L⁻¹, it had a significant effect on the concentration of chlorophyll in plants. This can be attributed to the fact that tryptophan is an indole-3-acetic acid (IAA) precursor, that causes cell growth and increases photosynthetic efficiency. [20] tryptophan initiates auxin biosynthesis, and magnesium enhances the photosynthetic machinery. together, these two produce more pigment to add up to, hence they are synergistic [21]. confirm that plant growth regulators and nanomaterials synergistically boost photosynthesis and plant productivity over either of them separately. Statistical evidence supports

the claim: that application of tryptophan had the most effect on the chlorophyll content in zinnia plants[22].

Table (3) shows that nano-magnesium and tryptophan had a remarkable influence on the carbohydrate content of *Zinnia elegans*. the effect of tryptophan supplementation was certainly positive, with the carbohydrate content rising progressively with increasing concentrations and reaching its maximum at 60 mg L⁻¹. The fact that tryptophan the activity of enzymes, and boosting protein development demonstrating that tryptophan is a key player in plant physiological process regulation by recording similar findings [23].nano magnesium had fewer yet favorable outcomes in terms of the amount of carbohydrates when compared to tryptophan [24]. Magnesium is involved in photosynthesis because it is a constituent of chlorophyll and its nano-formula promotes dissolution and increases its surface area. They discovered that nano-fertilizers enhance photosynthetic efficiency and nutrient uptake. [25] that nano-fertilizers and amino acids work together in the same manner.the results show that the combination of amino acids with nano-nutrients is effective in augmenting larger surface area, allowing it to be more easily absorbed by plants and carried into their the physiological efficiency and biochemical performance of ornamental crops like *zinnia elegans*, leading to higher yields. Results show a clear rise in the number of leaves as the concentration of nano-magnesium increases from 0 to 100 mg L⁻¹. Plants treated with 100 mg L⁻¹ had highest leaf numbers, followed by the control. magnesium is an important part of chlorophyll as it is needed for photosynthesis, enzyme activation, and passing ATP. nano-magnesium is more active and contains has a tissues [26]. This encourages plant the growth and leaf development. These findings support previous studies indicating that nano-fertilizers enhance plant productivity and nutrient uptake efficiency [27]. with 100 mg L⁻¹, The diameter of the flower increasedat 0 mg L⁻¹, showing that nano-magnesium had the most significant effect. This increase can be explained by the need for magnesium in photosynthesis and chlorophyll synthesis, which subsequently enhances carbohydrate accumulation and provides the energy required for flower initiation and growth [28] Tryptophan treatment had an intermediate influence; at 30 mg L⁻¹, there were 8.22 more flowers than in the control treatment, and at 60 mg L⁻¹, more flowers. Tryptophan promotes flowering by regulating cell division and elongation [29].

Exogenous tryptophan enhances sunflower reproductive and vegetative development by influencing auxin metabolism. when the concentration of tryptophan increased from 0 to 30 mg L⁻¹, N content increased the most,. It slightly declined at 60 mg L⁻¹ , though. From this information, it can be seen that the optimum quantity of tryptophan for nitrogen absorption is in the mid-range[30]. the improvement that was noted can be assigned to the role of tryptophan as promoting plant growth and increasing nutrient absorption in the plant as the concentration of nano-magnesium increased, the nitrogen content gradually increased from at 0 mg L⁻¹ to at 100 mg L⁻¹. Magnesium is the central atom of chlorophyll molecules, and is crucial for the initiation of enzymatic reactions initiating photosynthesis. Following the administration of nano-magnesium, the assimilation and metabolism of nitrogen improved, thus improving photosynthesis. If tryptophan and nano magnesium were treated concurrently, they proceed better. The value for N was the greatest at 60 mg L⁻¹ concentration of tryptophan and 100 mg L⁻¹ concentration of nano-magnesium, when the concentration was 30 mg L⁻¹ of tryptophan and 0 mg L⁻¹ of nano-magnesium, it **declined**, combining the use of growth regulators and nano fertilizers enhanced physiological functioning as a consequence of eminent utilization thus increasing the vegetative growth of the plant This is positively reflected on floral growth [31].

Conclusions

Research data confirms that *Zinnia elegans* responds positively to tryptophan at 100 mg/L or magnesium nanoparticles for foliar applications as evidenced by improved plant growth and flower parameters. The sole use of 100mg/L tryptophan resulted in the highest values for flower size and quantity, while still maintaining the strength and structure of the stem. When T9 treatment reached higher dosages with both tryptophan and use, it gave poor flower results, possibly because of the competitive effects of the chemicals. The use of magnesium nanoparticles at medium levels (30 -50 -mg/L) increased plant stem heights, but in concert with high tryptophan levels resulted in, poor floral outcomes. The growth stimulants that affect both tryptophan and magnesium nanoparticles can contribute to rhyme; attempting to combine both may require more control. Applying the right volume of biostimulants and nano-fertilizers adds value to ornamental plant production, provided that the method of application and frequency were calibrated.

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Effect Organic Fertilizers and Nano- NPK on Productivity and Quality of Fruits of Date Palm *Phoenix dactylifera* L., Cultivar Al-Khistawi

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Abstract. This experiment was conducted in one of the orchards of Babylon province- Al-Qasim District - Al-Amadiya Village during the period 2023-2024, To show the effect of adding compound fertilizer (N.P.K at 0, 0.5, 1 kg/tree) and Organic fertilizers (0, 5, 10 kg/tree) on the productivity and some fruit traits of date palm trees of the Al-Khistawi cultivar, where 27 palm trees were selected, aged 25 years, almost identical in size, and planted on lines and with planting distances of 9×9 m. A compound fertilizer of Jordanian origin and decomposed sheep waste were used. The study was conducted with three additions; the first on 1/11/2023, the second on 1/2/2024, and the third on 1/4/2024. The results of the experiment showed an increase in the total production, fruits weight, average number of bunches, fruits content sugar, percentage, and the percentage of each (bunches of nitrogen, phosphorous, potassium, and total carbohydrates), when the study factors used individually or in combination in the leaves) and the best results were obtained were when using a concentration of 1 kg of compound fertilizer and 10 kg of Organic fertilizers for all the mentioned traits..

Keywords. Chemical Fertilizer L, Date Palm Cultivar, Organic Fertilizer, Al-khistawi, *Phoenix dactylifera* L.

1. Introduction

in palm, (*Phoenix dactylifera* L.) belonged to the order Palmae and the family Arecaceae. The palm family is considered to be one of the oldest fruit trees in the world. The order Palmae is considered one of the Palmae cultivation spreads in tropical and semi-tropical regions; the Arabian Gulf region is its original homeland, and it moved to all areas with the appropriate atmosphere for its cultivation [1] and [2].

From an economic point of view, the palm occupies an important place in the agricultural sector in more than 40 countries in the world,[3] and in most cases, the lands of palm orchards are used in the cultivation of some types of fruit trees, vegetables, field crops, and ornamental plants. Palm orchards, date pressing operations, and industries derived from the vegetative and fruit parts of the palm also contribute to employing a certain percentage of workers in several countries

worldwide [4]. Iraq's date production for the 2021 season was estimated to be approximately 4,735 thousand tons. Baghdad province ranked first, followed by Babylon province in second place, and Diyala province ranked third. The production of the Al-Khistawi variety was estimated at approximately 396.7 thousand tons, and it is the most common cultivar in Iraq, and it constituting approximately 54 % of the date production in Iraq [5].

Most of the research focused on studying the components of the main product of the palm tree, which is dates, and the studies dealt with them in terms of their nutritional, medical and economic importance [6]. The element content of leaves and fruits is affected by the characteristics of the soil and the elements it contains, where increasing soil fertility through nitrogen, phosphate and potassium fertilization encourages the absorption of nutrients and increases the content of these elements in palm leaves, which is positively reflected in the quantity and quality of yield [7].

2. Materials and Methods

2.1. Experimental Site and Design

Twenty-seven trees of the Al-khistawi cultivar were selected, homogeneous in age and size, approximately planted on lines, and the distance between one tree and another was approximately 9 m. The experiment was conducted following the design of randomized complete blocks (R.C.B.D) as a factorial experiment (3×3). The compound fertilizer (NANO NPK with 0, 0.5, and 1kg/tree) and organic fertilizers (0, 5, and 10 kg tree) and the averages were compared using the least significant difference (L.S.D) test at a probability level of 0.05 [8]. The balanced compound fertilizer N.P.K 20:20:20 in the form of granules of Jordanian origin was used as a source of the compound fertilizer, as well as decomposed and dry sheep waste.

2.2. Studied traits

2.2.1. Average Tree Yield (kg)

This was calculated by calculating the rate of each treatment after the fruits reached the full maturity stage.

2.2.2. Average Weight of the Fruit (g)

It was calculated after collecting the fruits and taking 50 fruits from each treatment at random and weighing, and extracting the average.

2.2.3. Average Number of Bunches (bunch/ tree)

The number of tastes was calculated for each treatment and for three replicates, then the average was calculated.

2.2.4. Percentage of Total Sugars in Fruits %

Estimated according to the modified Lane and Eynon method [7].

2.2.5. Leaves Content of Nitrogen, Phosphorous, and Potassium %

Nitrogen content was estimated using the Kjeldahl method [8]. Total phosphorous was estimated using ammonium molybdate and a spectrophotometer according to the method described in [9], and potassium was estimated using a flame photometer according to the method proposed by [10].

2.2.6. Carbohydrate Content of Leaves (Bunches) (gm/kg Dry Weight)

The carbohydrate content of the leaves was estimated according to a previously described the method [11].

2.3. The Treatments

- Control (distilled water only).
- Compound fertilizer 0.5 kg / tree
- Compound fertilizer 1 kg / tree
- Organic fertilizers 5 kg/tree
- Organic fertilizers 10 mg/tree
- Compound fertilizer 0.5 kg / tree + Organic fertilizers 5 kg / tree
- Compound fertilizer 0.5 kg / tree + Organic fertilizers 10 mg / tree
- Compound fertilizer 1 kg/tree + Organic fertilizers 5 mg/tree
- Compound fertilizer 1 kg / tree + Organic fertilizers 10 mg / tree

Table 1. Some chemical properties of Organic fertilizers.

Electrical conductivity/DS.M	31.43
pH	6.9
Organic matter g/kg	234
Carbon g/kg	153
C/N	8.3
nitrogen g/kg	16.9
phosphorous g/kg	17.8
potassium g/kg	31

3. Results

3.1. Average Tree Yield (kg)

Table data (2) we see that the addition of the compound fertilizer led to a significant increase in the productivity of the tree, and the highest production rate reached (81.64 kg) was observed in trees treated with a concentration of 1 kg/tree compared to the lowest production amount (70.76 kg) in the control treatment. The addition of organic fertilizers increased the productivity of trees, which reached the highest production average when using the concentration of 10 kg/tree, and it was recorded at (74.95 kg) and less production (70.76 kg) in the control trees. As, and the interaction between the concentrations of compound fertilizer and organic fertilizers , was significant, where the trees treated with the highest concentration of each of them excelled by obtaining the highest yield (88.87 kg) compared to the lowest averages (70.76 kg) in the control treatment. The increase in the productivity of trees may be due to the effect of the compound fertilizer in filling the plant's need for the mineral elements necessary for photosynthesis, respiration, and various metabolic processes because the compound fertilizer contains primary elements.

3.2. Average Weight of the Fruit (g)

Of data in Table (3) we find the addition of the compound fertilizer caused increase in the average weight of the fruit, and he reached (10.51 gm) when adding 1 kg/tree. The lowest average weight was recorded (8.30 g).The addition of organic fertilizers increased the fruit weight average, which reached the highest average when using a level of 10 kg/tree, and it was recorded at (8.78 g) compared to the lowest averages (8.30 g) in the control trees. The interaction between

the concentrations of compound fertilizer and organic fertilizers , was significant, as the trees treated with the highest concentration of each of them excelled by obtaining the highest average fruit weight (11.24 g) compared to the lowest averages(8.30 g) in the control treatment.

3.3. Average Number of Bunches(bunch/tree)

In the table data (4) indicate that the addition of the compound fertilizer led to a significant increase in the average number of bunches on the tree, and the highest average reached (7.03 bunches/ tree) in trees treated with a concentration of 1 kg / tree compared to the lowest number of bunches, which is (5.68 bunches / tree) in the control treatment. The addition of Organic fertilizers also had a significant effect in increasing the number of bunches, which reached the highest averages when using the level of 10 kg / tree, and it was recorded at (6.01 bunch / tree) compared to the lowest average (5.68 bunches/ tree) in the control trees. As for the interaction between the concentrations of compound fertilizer and Organic fertilizers , it was significant, where the trees treated with the highest concentration of each of them excelled in obtaining the best results and amounted to (8.76 bunches/tree) compared to the lowest average (5.67 bunch /tree) in the control treatment.

3.4. Percentage of Total Sugars in Fruits %

The results in Table (5) confirm that the use of the compound fertilizer led to a significant in the percentage of total sugars, and the highest average reached (81.64%) in trees treated with a concentration of 1 kg/tree compared to the lowest percentage of total sugars (70.76%) in the control treatment. The addition of Organic fertilizers had a significant effect on increasing the percentage of total sugars, which reached the highest average for this trait when using a concentration of 10 kg/tree, and it was recorded at (74.95%) compared with the lowest average (70.76%) in the control trees. Also, the interaction between the concentrations of compound fertilizer and Organic fertilizers was significant, where the trees treated with the highest concentration of each of them excelled with the highest percentage of total sugars (88.87%) compared to the lowest average (70.76%) in the control treatment.

3.5. Leaves (Bunches) Content of Nitrogen %

As shown data in Table (6) the use of the compound fertilizer caused a significant increase in the nitrogen content of the leaves, and the highest average of this trait was reached (2.52%) when adding 1 kg/tree compared to the lowest averages of the carbohydrate content in the leaves when the comparison in which the leaf content decreased From Nitrogen to (1.530%) The addition of Organic fertilizers had a significant effect in increasing this trait, and the largest rate when using 10 kg/tree, and it was recorded at (1.873%) compared with the lowest averages (1.530%) in the control trees. and the interaction between the concentrations of compound fertilizer and Organic fertilizers , it was significantly to increase the nitrogen content of the leaves, where the trees treated with the highest concentration of each of them excelled with an average of (2.532%) compared to the lowest averages (1.530%) in the control treatment.

3.6. The Percentage of Potassium %

The data in Table (8) indicate that the use of the compound fertilizer caused increase in the potassium, and the highest average for this trait reached (2.252%) when adding 1 kg/tree compared to the lowest averages of potassium in leaves when compared to the comparison in which the leaf content decreased from potassium to (1.530%).The addition of Organic fertilizers had a significant effect on increasing this trait, and the highest average was when using at 10 kg/tree, and it was recorded at (2.179%) compared with the lowest averages (1,873%) in the

control trees. As for the interaction between the concentrations of compound fertilizer and Organic fertilizers , it was significantly to increase the potassium content of leaves, where the trees treated with the highest concentration of each of them excelled with an average of (2.532%) compared to the lowest average (1.530%) in the control.

3.7. Carbohydrate Content of Leaves (Bunches) (g/kg Dry Weight)

The data in Table (9) the use of compound fertilizer caused a significant increase in the carbohydrate content of the leaves. The highest average of this trait was (4.531 g/kg dry weight) when adding 1 kg/tree compared to the lowest averages of the carbohydrate content of leaves when compared to which the carbohydrate content of leaves decreased to (2.917 g/kg dry weight). The addition of Organic fertilizers had a significant effect on increasing this trait, and the highest rate was of 10 kg/tree, which was recorded at (4.537 g/kg dry weight) compared to the lowest averages (2.917 g / kg dry weight) in the control trees. As for the interaction between the concentrations of compound fertilizer and Organic fertilizers , it significantly increased the carbohydrate content of leaves, where the trees treated with the highest concentration of each of them excelled with an average of (4.813 g/kg dry weight) compared to the lowest averages (3.537 g/kg dry weight) in the control treatment.

Table 2. Effect of adding compound fertilizer (N-P-K) and Organic fertilizers on productivity and some traits of fruits and leaves of date palm *Phoenix dactylifera* L, cultivar Al- khistawi

Organic fertilizers concentration kg/tree	Compound fertilizer concentration kg/tree	CH O	fruit/g m	N	Number of bunches	Total sugars in fruits %	Tree productivity/kg	Potassium (g/kg dry weight)
0	0	2.917	8.30	1.34	5.68	65.46	70.76	1.530
	0.5	3.862	8.91	1.14	6.31	69.07	76.64	2.0231
	1	4.531	10.51	2.53	7.03	72.58	81.64	2.052
5	0	3.217	8.77	2.12	5.67	67.18	74.05	1.865
	0.5	3.980	9.02	2.21	6.67	71.74	76.66	2.055
	1	4.760	10.78	2.55	7.32	73.06	83.91	2.400
10	0	3.537	8.78	2.20	6.01	67.94	74.95	1.873
	0.5	4.243	10.24	2.24	7.11	72.16	81.35	2.179
	1	4.813	11.24	2.80	8.76	74.16	88.87	2.532
L.S.D.	Organic fertilizers concentration kg/tree	1.367	0.672	0.584	0.535	1.039	3.297	0.0284
(P≤0.05)	Compound fertilizer concentration	1.367	0.672	0.584	0.535	1.039	3.297	0.0284

	kg/tree						
Interaction	2.73 4	1.344	1.16 8	1.007	2.078	6.594	0.0568

4. Discussion

The increase resulting from applying the organic fertilizer can be attributed to the fact that the microorganisms present in the fertilizer secrete chemical substances known as siderophores. These compounds chelate essential nutrients particularly potassium, phosphorus, and nitrogen—making them more readily available to the plant. This enhances nutrient absorption and accumulation within plant tissues, thereby improving the plant's nutritional status. Consequently [12]. vegetative growth is promoted through increased leaf area and chlorophyll content, which enhances the plant's photosynthetic capacity, leading to carbohydrate accumulation and a higher percentage of dry matter. Ultimately, this results in a greater yield [13]. These findings align with those of Hasaballah The resulting increase in the studied characteristics due to the addition of nano-fertilizer may be due to the direct effect of nano-NPK as a result of the smooth and direct absorption of nutrients by the roots and thus its positive effect on the chlorophyll content, as nitrogen enters the construction of the porphyrin ring, which has an essential role in building chlorophyll [14]. It also increases auxins and cytokinins, which increases cell elongation, expansion, and division, and increases the readiness of amino and nuclear acids [15]. Plastids contain 78% of the total nitrogen present in the plants. Phosphorus plays an important role in the formation of both amino acids and essential proteins in building plastids and in stimulating the growth of cell walls, promoting vegetative growth, increasing the ability of the roots to absorb nutrients, and accumulating marginal substances in the leaves as a result of increased photosynthesis [16]. In addition to the role of potassium, which activates many enzymes, it has a direct role in the formation of enzymes involved in the formation of plastids and in increasing physiological processes such as photosynthesis and respiration, in addition to activating metabolic processes that activate cell division, elongation, and expansion [17]. By increasing K, the strength of vegetative growth increases because it strengthens cell walls. It also plays an important role in the mechanism of opening and closing stomata, as well as in activating enzymes for photosynthesis. Increasing the power of photosynthesis and the accumulation of its products inside the plant leads to an increase in growth power, which is reflected in yield [18]. Our results showed an increase in the indicators of the studied characteristics due to the addition of nano-fertilizer, which was reflected in the characteristics of vegetative growth and yield, and this agrees with on the palm trees of the Khastawi variety and [18] on the palm trees indicating increased vegetative growth of the mentioned characteristics. [19] As for the increase as a result of using the biofertilizer, the reason may be due to the biofertilizer containing in its composition bacteria that encourage photosynthesis and lactic acid bacteria, in addition to yeasts and fungi that increase the growth strength of the plant [20]. It plays a major role in secreting compounds that chelate some nutrients, such as iron, manganese, and phosphorus, which leads to their accumulation in the soil and thus increases their content inside the plant, which affects growth and yield [21]. Or it may be due to the fact that the organisms present within the fertilizer have the ability to produce compounds similar to auxins and gibberellins that work to stimulate cell division and elongation and increase the readiness of the elements, which is reflected in increased growth of roots and shoots and an increase in the effectiveness of photosynthesis, which is reflected in the strength of the plant's shoots and thus increases its content of Dry matter and all of this is reflected positively on the outcome [22]. This agrees with foron the palm variety Barhi and [23] foron the shlek plant. As for Tthe increase resulting from treatment with algae extract,

the reason may be due to its content of nutrients such as nitrogen, potassium, and phosphorus, in addition to their content of vitamins and auxins that have a direct or indirect role in plant metabolism,[25]and[26]. so vegetative growth increases and the leaf's chlorophyll content increases, which is reflected in the structure. Photosynthesis, which leads to the accumulation of carbohydrates and thus all of, this is reflected in the yield and its characteristics. This is in agreement with for on palm trees [27] and [28].

Conclusions

It was shown from the above that adding 1, kg/tree (NANO NPK) (F2) excelled in most of the studied traits. It also showed that adding ,(10 kg tree) (E1) Organic fertilizers significantly superior in growth traits and yield.. The double and triple interaction between the study factors had a major role in improving the strength of vegetative growth, which reflected positively on the yield characteristics.

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The Effect of Spraying with Nano-Proline, Nano-Glutathione, and Nano-Ascorbic Acid on Oxidation Enzymes and *FaGPX* Gene Expression in Strawberries Irrigated with Well Water

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Abstract. In this study, we investigated the effects of spraying proline at concentrations of 0, 200, and 400 mg L⁻¹, as well as the antioxidant glutathione (0, 100, and 200 mg L⁻¹) and ascorbic acid (0, 50, and 1100 mg L⁻¹), on certain chemical indicators of strawberry cultivar Ruby Gem seedlings irrigated with salty well water. The experiment was conducted in the Horticulture and Forestry Division of the Najaf Agriculture Directorate between November 17, 2024, and April 1, 2025. A completely randomized block design was used to create the factorial experiment. Nano-proline was the first factor, followed by nano-glutathione, and nano-ascorbic acid. Consequently, there were 81 experimental units in total, with 27 treatments randomly assigned to three blocks. The GenStat statistical analysis tool was used to examine chemical trait rates, and the L.S.D. test was used to compare the average findings at a 5% probability level. The most significant findings seemed to indicate that nano-proline acid significantly impacted certain molecular and chemical markers of the strawberry plant, outperforming the following traits: ascorbic peroxidase enzyme at a concentration of 0 mg.L⁻¹ if it reached (0.918 absorption units g⁻¹) and peroxidase enzyme at a concentration of 200 mg.L⁻¹ gave 2.65 IU g⁻¹ fresh weight. When the antioxidant nano-glutathione was sprayed at a concentration of 0 mg.L⁻¹, the ascorbic peroxidase enzyme significantly increased, yielding 1.111 absorption units g⁻¹. After being sprayed with nano-ascorbic acid at a dosage of 0.1 mg L⁻¹, the ascorbic peroxidase enzyme's characteristic rose to 1.111 absorption units g⁻¹. The reciprocal relationships between the factors attained that the majority of the chemical and molecular markers in the three trials responded significantly. The rate of peroxidase activity and overall activity of ascorbic peroxidase in the leaves were significantly affected by the triple interaction among the three experimental conditions. These characteristics increased when the antioxidants nano-proline, nano-glutathione, and nano-ascorbic acid were not sprayed, as evidenced by the following results: 6.83 IU g⁻¹ fresh weight and 1.720 absorption units g⁻¹. The experimental factors affected the relative amount of gene expression for the *FaGPX* gene, as the concentrations of proline, glutathione, and ascorbic acid were reducing factors in gene expression for the *FaGPX* gene under salt stress conditions when irrigated with saline water.

Keywords. *FaGPX* Gene expression, Nano- proline, Nano-glutathione, Nano-ascorbic acid, Well water.

1. Introduction

Strawberries (*Ananassa* Duch. $\times$ *Fragaria*) belongs to the Rosales order, Rosaceae family, Rosoideae subfamily, and *Fragaria* genus, which includes 45 species. It is a hybrid of Virginia and Chilean strawberries [1]. Strawberries are produced in 63 countries, with global production reaching 10,485,454.06 tons annually, with China ranking first globally, followed by the United States. In Iraq, the average productivity has reached 18.89 tons [2], as its cultivation is concentrated in large areas in the governorates of Baghdad, Diyala, and Salah al-Din, while the cultivated area is smaller in Wasit and Karbala. Baghdad Governorate had the largest total productivity, reaching 8.21 tons, followed by Salah al-Din Governorate, with a productivity of 8.18 tons [3]. Gene expression analysis technology, in addition to being able to distinguish between individuals, is a method for the early detection of plant diseases introduced through airports and research centers. It is used to control the quality of introduced plant material, detect its products, and distinguish between plant varieties and species. DNA-Sequencing is one of the most important molecular technologies because it has become possible to accurately identify the nucleotide sequence of a specific DNA fragment. Adults, and there is no doubt that this technology has added a new dimension to molecular biology and revealed many of the secrets surrounding the DNA molecule. It has also made it possible to identify the nature of many complex genes and their properties [4]. Salinity is a major abiotic factor that negatively affects the growth and development of plants. Salinity causes osmotic stress and ionic imbalance, leading to metabolic disturbances, nutrient imbalance, and membrane dysfunction, severely affecting plant morphology and physiological processes [5]. Free radicals generated when plants are subjected to biotic and abiotic stressors are scavenged by proline, an antioxidant [6]. Microorganisms, plants, and mammals contain the antioxidant glutathione. It can prevent reactive oxygen species (ROS), such as heavy metals, lipid peroxides, free radicals, and peroxides from damaging vital cellular components. Glutathione is essential for plant survival. Researchers have focused on glutathione in recent years in an effort to better understand how it affects plants under stress, raises its concentration, and improves plant resistance to stress [7]. It is a non-enzymatic antioxidant that can limit or reduce the harmful effects of reactive oxygen species (ROS) produced by various plant metabolic processes. Thus, acid protects cell membranes from damage and maintains the osmotic potential and ionic balance in cells [8]. Ascorbic acid is an important cofactor in the synthesis of many plant hormones, including ethylene and abscisic acid (ABA) [9,10].

2. Materials and Methods

The experiment was carried out on Strawberry (Ruby Gem Cv.) seedlings propagated by incubators as a factorial experiment within a Randomized Complete Block Design (RCBD). The experiment includes three factors: the first factor is treating the seedlings with Nano-proline at a concentration of (0, 200, 400 mg L⁻¹). The second factor is spraying the seedlings with Nano-glutathione acid at a concentration of (0, 50, 100 mg L⁻¹). The third factor is spraying the seedlings with Nano-ascorbic acid at a concentration of (0, 100, 200 mg L⁻¹). There are three replicates (sectors). Each treatment within each sector contains six (experimental units), so that the total number of seedlings is 486, in addition to seedlings as guard lines on both sides of the sectors. The planting lines are planned and prepared in the greenhouse in three lines, leaving a distance of 1 m between each line and the planting distance between plants is 25 cm. The soil was analyzed in the laboratory of the Directorate of Agriculture in Najaf Governorate to measure its physical and chemical properties, and the results were as shown in Table (1).

Table 1. Soil analysis.

Property	Value	Unit of Measurement
Soil Separates	Sand 58.2	%
	Silt 25.0	
	Clay 16.8	
Soil Texture	Sandy Loam	----
pH	6.7	
EC	3.9	dS/m ⁻¹
CO ₃	Nil	
N	0.361	PPm
P	0.345	PPm
K	92.2	PPm
Ca	4	mmol·L ⁻¹
Mg	9.6	mmol·L ⁻¹
Cl	5	mmol·L ⁻¹
HCO ₃	0.7	mmol·L ⁻¹
SO ₄	0.42	mmol·L ⁻¹
Organic Matter	0.1	%

The irrigation methods used prior to the experiment were randomly sampled and planted in greenhouses in agricultural rows covered with black plastic. Soil mulch was sprayed every month after the appearance of the fully developed true leaves. Regarding the type of water used (well water), spraying was carried out in the early morning until the vegetative mass was wet, with the addition of a spreading agent (Tween 20) at a rate of 0.1%. The results were analyzed by ANOVA using the GeneStat statistical program, and comparisons between treatments were made using the Least Significant Difference (LSD) test at a probability level of 5% [11].

2.1. Enzyme Activity Assessment

2.1.1. Assessment of the Total Peroxidase Activity (POX) (International Units/g⁻¹ Fresh Weight

Peroxidase activity was measured using a previously described procedure [12]. Resources and Analysis Employed Guaicaol Solution: Made by combining 1.36 milliliters of guaicaol with 250 mL of distilled water in a volumetric flask. 0.1% Hydrogen Peroxide Solution (H₂O₂): Made by adding Distilled water to 120 ml of 0.4 ml of 30% H₂O₂. The total peroxidase activity: Combine one milliliter of guaiacolate with one milliliter of hydrogen peroxide (H₂O₂). The samples were then centrifuged at 12,000 rpm for 15 min. Following their coding and numbering in accordance with the parameters, the samples were subsequently allocated to spectrophotometer tubes. The absorbance was measured at 420 nm using a spectrophotometer. Add 2 milliliters of the reaction mixture and then 0.1 milliliters of the sample to a cell in a Japanese Spectrumlab 22 to estimate the total peroxidase activity. At a wavelength of 420 nm, the change in light absorbance is measured every 30 seconds for three minutes.

$$\text{Enzyme Activity (unit/mg protein)} = (\Delta A / \text{minute} \times V_t \times 1000) / (\epsilon \times V_s \times \text{protein})$$

$\Delta A / \text{minute}$: Change in absorbance per minute, V_t : Total reaction volume (3.1 ml), V_s : Extract volume (0.1 ml), ϵ : Extinction coefficient (26.6 mM⁻¹cm⁻¹ for tetraguaiacol), protein: Protein concentration in the extract (mg/ml)

2.1.2. Estimation of Ascorbate Peroxidase Activity (Unit)

The activity of ascorbate peroxidase (APX) was estimated according to a previously described method [13], where the following solutions were prepared: Preparation of 0.1 M potassium phosphate buffer solution (K-buffer) and 1.3609 g of KH_2PO_4 (molecular weight 136.09 g/mol) were dissolved in 100 ml of distilled water (acidic solution), 1.7418 g of K_2HPO_4 (molecular weight 174.18 g/mol) was dissolved in 100 ml of distilled water (basic solution), and a sufficient quantity of KH_2PO_4 was taken for measurements, and its pH was adjusted to 7 by adding K_2HPO_4 . 2) Prepare a 0.025M ethylenediaminetetraacetic acid (EDTA) solution (molecular weight 292.25 g/mol) by dissolving 7306.0 g of EDTA in 100 ml of distilled water. The pH was adjusted to 8 by adding sodium hydroxide (0.5 M) to completely dissolve EDTA in distilled water. Prepare a 0.01 M ascorbic acid solution (molecular weight 176.12 g/mol) was prepared by dissolving 0.0881 g of ascorbic acid in 50 ml of distilled water. A hydrogen peroxide solution (H_2O_2) was prepared. Dissolve 4 ml of 50% H_2O_2 in 100 ml of distilled water.

The procedure was that: Mix 1.5 ml of k-buffer containing ascorbic acid (0.5 ml of ascorbic acid, 0.2 ml of EDTA, and 0.2 ml of H_2O_2). Add 0.1 ml of the enzyme extract, making a total volume of 2.5 ml. The difference in absorbance was measured using a spectrophotometer at a wavelength of 290 nm.

The enzyme concentration was calculated using the following equation:

$$\text{APX activity}(\text{unit}) = (\Delta\text{Abs} / \text{min} \times \text{reaction volume}) / (2.8 \text{ mM}^{-1})$$

2.2. Molecular Characteristics

2.2.1. Collection of Plant Samples

Molecular markers were measured using the Wahj DNA Genomics Laboratory/Baghdad Al-Jadriya. Leaf samples were collected from the Ruby Gem variety of strawberries for each treatment in three replicates on 4/25/2025 to measure the relative amount of gene expression of the gene for the production of the enzyme (glutathione peroxidase) symbolized by (*FaGPX*) in the Ruby C variety of strawberry under the influence of experimental factors using Realtime-PCR technology.

2.2.2. Preparation of Plant Samples

Leaves free from disease were selected, and samples were placed in tightly closed nylon bags, which were marked with indicative symbols. The samples were then transferred to a laboratory. The leaf samples were washed several times with sterile distilled water to remove dust and particles. They were then wiped with cotton wool soaked in 70% alcohol for sterilization. They were then cut into small pieces (1 cm^2) using clean sterile sharp scissors. The pieces were then placed in a ceramic mortar, and liquid nitrogen was added very carefully and with extreme caution to avoid the damage caused by nitrogen when it comes into contact with the skin or any visible organs of the body. The samples were then ground well until they turned into a white powder. The powder was stored in a 10 ml storage tube, and the samples were stored in a laboratory refrigerator until they were used for measuring gene expression or molecular characterization.

2.2.3. Design of Study Primers

Primers were designed using the NCBI website based on bioinformatics, and the appropriate primer was chosen based on the standard specifications of the primer to measure the relative amount of gene expression or molecular characterization.

2.2.4. Preparation of Primers

The primers used in this study were prepared by Bioneer (South Korea). The primers contained two parts (reverse and forward) and included primers for quantitatively measuring the expression of the glutathione peroxidase gene (*FaGpx*) (KC433891.1) (F/ 5-CGGTTTCCCGTGCAATCAG T-3) and (R/ 5-ACACCGGGGACCAAATGATGATG T-3) in the Ruby Gem Cv Strawberry cultivar as well as the actin gene (*Faβ-ACTIN*), which was used as a reference gene (housekeeping gene) (F/ 5- GGCATCCTGACCCTGAAGTA -3) and (R/ 5- GGGGTGTTGAAGGTCTCAAA -3). Primers were prepared using deionized water (DDW) and fixed according to the manufacturer's recommended primer concentration. The primer mix tubes were prepared as follows: Forward (10 µL) + reverse (10 µL) + DD.W (80 µL) = 100 µL primer mix.

2.2.5. Total RNA Extract

Total RNA was extracted from the leaves of the Ruby Gem variety of the treated strawberry using (Sv Total RNA isolation kit), according to the method [14], based on filters and extraction solutions and using the following materials, tools, and devices, as shown in table (2).

Table 2. Total RNA isolation kit contents.

RNA isolation kit	RXn
RNA Lysis Buffer + (Ethanol)	500 µl
RNA Dilution Buffer	350 µl
RNA Wash Solution	600 µl + 600 µl + 250 µl
DNase Stop Solution	200 µl
Nuclease – Free water	100 µl

2.2.6. Agarose Gel Electrophoresis of RNA, cDNA, or RT-PCR Products

Agarose gel electrophoresis was used to verify and test the extraction of total RNA and cDNA, and to test the success of cDNA synthesis using reverse transcriptase (RT-PCR) from strawberry leaves (table 3). The plate was lifted from the basin and placed in an imaging device connected to an electronic computer (gel documentation station) according to a special program for imaging gels (Gel Doc exp.). The sections became clear and the molecular sizes of the bundles were estimated based on the thickness of the size indicator on the side of the gel [15]. table (3).

Table 3. Agarose gel electrophoresis contents.

Materials	Contents	Mount
TBE Buffer (10x) Dilution 10 time to get 1X	Tris Base	108 g / 1 L
	EDTA (0.5 M) pH 8	40 ml / 1 L
	Glacial acetic acid	55 g / 1 L
	Distilled water (D.W)	800 ml
Loding dye	For 1 L	
	Bromophenol blue	0.25 g / 100 ml
	Glycerol	30 ml / 100 ml
Agarose (1%)	Distilled water (D.W)	25 ml (100 ml)
Ethidium Bromede- Red Safe	Nucleic staining solution	1 g / 100 ml (TAE 1%)
DNA marker (M)	DNA Lamida phage fragments	3 µl/ 100 ml (TAE 1%)
		5 µl

2.2.7. cDNA Synthesis

cDNA was synthesized using a kit (Power cDNA Synthesis kit) according to the method described in [16]. The cDNA Master Mix was prepared, the kit contents were mixed with distilled water (D.W.), and RNA samples were extracted from treated Ruby C Strawberry leaf samples, according to the reaction shown in table (4).

Table 4. Master Mix for cDNA Synthesis.

Master Mix	1 R X n (μ l)	18 R X n (μ l)
AMV Enzym	4	
RT Buffer (5 x)	10	180
Oligo(dT) ₁₅ Prim	5	90
DNTPs	5	90
D.W	10	180
Total	34 μ l	
RNA	16	
	50	

cDNA synthesis was tested by migration on 1% agarose gel.

2.2.8. Estimation and Analysis of the Relative Quantity of Glutathione Peroxidase (FaGPX) Gene Expression

Glutathione Peroxidase (FaGPX) gene expression was measured and analyzed using a Quantitative Real-Time PCR (QRT) device. SYBr Green dye was used using the Go Taq Master Mix SYBr Green PCR Kit (Promega) with FaGPX primers. The (H.k) *FaACTIN* primer was used as a reference gene for the relative measurement of gene expression (table 5).

Table 5. Master mix SYBr Green kit contents.

Master Mix	1 R x n (μ l)	54 R x n (μ l)
1- Go Taq master mix SYBr Green kit		
A- 100 mM KCl		
B- 40 mM Tris – HCl (pH 8.4)		
C- 0.4 mM dNTPs	12.5	675
D- U/ μ l I Taq DNA polymerase		
E- mM MgCl ₂		
2- Primer Mix (F + R + D.W)	2.5	135
3- Distilled water (D.W)	7.5	405
	22.5	1.215

The critical threshold for measuring gene expression was determined using two types of blank control assays: the first was a control treatment lacking cDNA (-cDNA) 1B, and the second was lacking primer B2 (-Primer). The calculation was repeated three times for each treatment, as well as for both the control treatments. This technique was employed to analyze the relative levels of gene expression. We created 54 cDNA tubes that were labelled in accordance with the treatment tubes. Each tube should contain 7.5 μ l of cDNA, with three replicates. Next, distribute 2.5 μ l of cDNA was distributed to the replicates in reaction plate tubes (plate tubes) that were placed within the apparatus. To display the types and control settings, prepare and mark a plate for real-time PCR equipment. Create a map that resembles the plate used in the live reaction. 25 μ l of the reaction material—either MixBlank SYBr Green 22.5 μ l or cDNA 2.5 μ l + M.Mix SYBr Green

22.5 μL —is contained in each reaction plate tube. The ready-mix tube with 675 μL of Go Taq Master Mix SYBr Green kit after preparing the SYBer Green Master mix. A primer mix (135 μL) was prepared and added to the collection tube. The tube was placed in a centrifuge set at $12,000 \times g$ for 6 s. After adding 405 μL of distilled water (DW) to the collection tube, it was centrifuged for 6 s at $12,000 \times g$. Three duplicates of each of the two control treatment types—Blank Master Mix—were produced, and water was added to complete the volume. After thoroughly mixing the master mix using a Microcentrifuge device for 4 s at a speed of $12,000 \times g$, the contents were divided at a rate of 22.5 μL into each tube of the real-time reaction plate. With the exception of the control treatment, which used incomplete cDNA (B-cDNA) and three duplicates of each type on a real-time PCR reaction plate, (2.5 μL) of cDNA was added to each tube until the volume in the reaction plate tubes reached (25 μL). The contents of the two blank control treatments were distributed according to the map of their location in the reaction plate of the plate device. The plate was covered with a transparent cover, called a Sealing Sheet, and adhered well. Avoid touching the transparent surface with one's hand. The reaction plate was placed in the real-time PCR device, and the reaction was monitored via a calculator screen connected to the real-time device and equipped with a special program (CFX Manager software). The program was set by selecting the program using Master, selecting the samples using Plate set up, which shows the plate, and by shading the sample according to the map and writing the name of the sample to be compared with the actin primer. The protocol, names, and colors of each sample were determined. The device was monitored until the lid temperature reached 103°C and the block temperature reached 72°C . This process was continued for 2.43 hours. Then, the charts start to appear, starting from the appearance of the critical line (C_t) threshold. The readings were automatically taken to the GeneX statistical program for comparison, and the quantity was measured and the data for the gene expression of the glutathione peroxidase gene used in the study of the Strawberry Ruby Gem variety [17].

3. Results

3.1. Chemical Indicators

3.1.1. Peroxidase Enzyme Activity Rate - POX (International Units/ g^{-1} Fresh Weight)

As shown in Table (6), spraying strawberry plants with the amino acid Nano-proline had a significant effect on peroxidase enzyme activity. For the first season, the Nano-proline concentration of 200 mg L^{-1} produced the highest rate of 3.50 IU/g^{-1} Fresh Weight, whereas the concentration of 400 mg L^{-1} produced the lowest rate of 2.65 IU/g^{-1} Fresh Weight. Additionally, the activity of the peroxidase enzyme in the first season was not significantly affected by the administration of the antioxidants nano-glutathione or nano-ascorbic acid alone. Table (6) shows that the rate of peroxidase enzyme activity was significantly affected by the dual interaction between spraying with Nano-proline and concentrations of the antioxidant nano-glutathione. The treatment (0 mg L^{-1}) increased the activity of the peroxidase enzyme in the leaves, reaching 4.77 IU g^{-1} fresh weight, in contrast to the spraying treatment (nano-proline 400 mg L^{-1} + nano-glutathione 200 mg L^{-1}), which decreased the percentage of peroxidase enzyme in the leaves, reaching 2.17 IU g^{-1} fresh weight for the first season. Plants sprayed with 400 mg L^{-1} nano-proline + 100 mg L^{-1} nano-ascorbic acid had the highest rate of peroxidase enzyme activity in the leaves, reaching 4.99 IU g^{-1} fresh weight. In contrast, plants sprayed with 400 mg L^{-1} nano-proline + 100 mg L^{-1} nano-ascorbic acid had the lowest percentage (1.63 IU g^{-1} fresh weight) in the first season. This indicated that the dual interaction between spraying Nano-proline and nano-ascorbic acid had a significant impact on this trait. Significant variations in the peroxidase

enzyme activity rate were also observed when nano-glutathione and nano-ascorbic acid were sprayed together. Compared to the spraying treatment with nano glutathione (200 mg L^{-1}) and nano ascorbic acid (100 mg L^{-1}), which recorded the lowest percentage (2.63 IU g^{-1} fresh weight) for the first season, the interaction ($200 \text{ mg L}^{-1} + 100 \text{ mg L}^{-1}$) recorded the highest percentage (4.10 IU g^{-1} fresh weight).

The spray treatment with nano-amino acid proline (0 mg L^{-1}), nano-antioxidant glutathione (0 mg L^{-1}), and nano-ascorbic acid (0 mg L^{-1}) increased the activity of the peroxidase enzyme in the leaves to 6.83 IU g^{-1} fresh weight, and the triple interaction between the three experimental factors demonstrated a significant effect on the rate of peroxidase enzyme activity in the leaves. In contrast, the spray treatments of nano proline (0 mg L^{-1}), nano antioxidant glutathione (0 mg L^{-1}), and nano ascorbic acid (100 mg L^{-1}) recorded the lowest rate of peroxidase enzyme in the leaves, reaching an activity of (1.35 IU g^{-1} fresh weight) for the study season. The interaction ($0 \text{ mg L}^{-1} + 50 \text{ mg L}^{-1} + 100 \text{ mg L}^{-1}$) recorded 6.07 IU g^{-1} fresh weight.

Table 6. The effect of spraying with Nano- proline, Nano- glutathione, and Nano-ascorbic acid, and their interaction on the activity rate of the enzyme peroxidase (POX) (International unit g^{-1} fresh weight) in strawberry plants, Ruby Gem Cv., irrigated with well water during the 2024-2025 study season.

Prolin(mg.L^{-1})	Ascorbic (mg.L^{-1})	Glutathion(mg.L^{-1})			
		0	100	200	Prolin x Ascorbic
0	0	6.83	6.07	2.06	4.99
	50	4.41	2.03	2.00	2.81
	100	2.31	2.54	2.88	2.58
200	0	3.23	3.28	3.74	3.42
	50	3.25	2.47	2.78	2.83
	100	2.77	5.59	3.84	4.07
400	0	3.32	3.73	5.49	4.18
	50	3.69	3.41	3.24	3.45
	100	1.95	1.60	1.35	1.63
Glutathion		3.44	3.49	3.04	
Glutathion mg.L^{-1}					
Glutathion x Prolin		0	100	200	Prolin
Prolin mg.L^{-1}	0	4.77	4.40	2.31	2.83
	200	4.61	2.45	3.45	3.50
	400	3.08	2.69	2.17	2.65
Ascorbic mg.L^{-1}					
Glutathion x Ascorbic		0	50	100	Ascorbic
Glutathion mg.L^{-1}	0	4.10	3.78	2.67	3.52
	100	3.97	2.63	3.76	3.45
	200	2.99	3.41	2.63	3.01
L.S.D. 0.05					
1.082 L.S.D Glutathion=					
L.S.D Prolin=1.082					
L.S.D Ascorbic =1.082					
1.874 L.S.D Glutathion x Prolin =					
L.S.D Glutathion x Ascorbic =1.874					
L.S.D Prolin x Ascorbic =1.874					

Prolin(mg.L ⁻¹)	Ascorbic (mg.L ⁻¹)	Glutathion(mg.L ⁻¹)			
		0	100	200	Prolin x Ascorbic
3.245		L.S.D Glutathion x Prolin x Ascorbic =			

3.1.2. Estimation of the Total Activity of Ascorbic Peroxidase (APX) (absorption unit g⁻¹)

It is noted from the results in Table (7) that not spraying with the amino acid Nano-proline has a significant effect on the total effectiveness of the enzyme ascorbic peroxidase, as it recorded the highest enzyme effectiveness of (0.918 absorption units.g⁻¹), compared to the treatment of spraying with Nano-proline (400 mg L⁻¹), which recorded the lowest effectiveness of the enzyme ascorbic peroxidase, as it reached (0.693 absorption units.g⁻¹) for the first season. The treatment of not spraying the strawberry plants with the antioxidant nano-glutathione had a significant effect, as it resulted in an increase in the total activity rate of the enzyme ascorbic peroxidase in the leaves, which reached (1.111 absorption units.g⁻¹), compared to the treatment of spraying with nano-glutathione (200 mg L⁻¹), which gave the lowest rate of the total activity of the enzyme ascorbic peroxidase in the leaves, which reached (0.671 absorption units.g⁻¹) for the first season. It is noted from the same table that no spraying of the strawberry plants with nano-ascorbic acid had a significant effect on the activity of the enzyme ascorbic peroxidase, as it gave the highest rate (0.923 absorption units.g⁻¹), compared to the treatment with nano-ascorbic acid (100 mg L⁻¹), which gave the lowest rate (0.619 absorption units.g⁻¹) for the first season.

It is noted from Table (7) that the two-way interaction between the concentrations of non-sprayed Nano-proline and the concentrations of the antioxidant nano-glutathione had a significant effect on the total activity rate of the enzyme ascorbic peroxidase, which led to an increase in the total activity of the enzyme ascorbic peroxidase in the leaves, which reached (1.099 absorption units.g⁻¹) for the study season, compared to the treatment of spraying with Nano-proline at a concentration of (400 mg L⁻¹) and nano-glutathione (200 mg L⁻¹) to reduce the total rate of the enzyme ascorbic peroxidase in the leaves, which reached (0.649 absorption units.g⁻¹) for the first season.

Table (7) shows that the two-way interaction between not spraying with Nano-proline and nano-ascorbic acid had a significant effect on the studied trait, as it gave the highest effectiveness, reaching (1.031 absorption units.g⁻¹) compared to the treatment with Nano-proline (400 mg L⁻¹) and ascorbic acid (100 mg L⁻¹), which recorded the lowest effectiveness of the ascorbic peroxidase enzyme in the leaves, reaching (0.522 absorption units.g⁻¹).

The interaction between non-spraying with the antioxidant nano-glutathione and nano-ascorbic acid was significantly superior, recording the highest activity of the APX enzyme (0.982 absorption units g⁻¹), whereas the interaction (50 mg L⁻¹ + 200 mg L⁻¹) was superior, with the highest weight (0.964 absorption units g⁻¹), compared to the lowest activity in the interaction plants between nano-glutathione (200 mg L⁻¹) and nano-ascorbic acid (100 mg L⁻¹) which reached (0.510 absorption units g⁻¹) in the first season.

The triple interaction between the three experimental factors significantly affected the total activity rate of ascorbic peroxidase in the leaves. The treatment of not spraying with nano-proline, not spraying with the antioxidant nano-glutathione, and not spraying with nano-ascorbic acid at a concentration led to an increase in the total activity of the enzyme ascorbic peroxidase in the leaves, which reached (1.720 absorption units.g⁻¹) for the study season, while the treatment of spraying with nano-proline (400 mg L⁻¹), spraying with nano-glutathione (200 mg L⁻¹), and spraying with ascorbic acid (100 mg L⁻¹) resulted in a low rate of the total activity of the enzyme ascorbic peroxidase in the leaves, which reached (0.229 absorption units.g⁻¹) for the study seasons.

Table 7. The effect of spraying with Nano- proline, Nano- glutathione, and Nano-ascorbic acid, and their interaction on the activity rate of ascorbic peroxidase (APX) (absorption unit g^{-1}) in strawberry plants, Ruby Gem Cv., irrigated with well water during the 2024-2025 study season.

Prolin $mg.L^{-1}$	Ascorbic $mg.L^{-1}$	Glutathion $mg.L^{-1}$			Prolin x Ascorbic
		0	100	200	
0	0	1.720	0.701	0.671	1.031
	50	1.009	0.794	0.734	0.846
	100	0.650	1.211	0.602	0.821
200	0	1.137	0.701	0.598	0.812
	50	0.836	0.630	1.058	0.841
	100	0.937	0.724	0.570	0.744
400	0	1.014	0.736	0.632	0.794
	50	0.970	0.671	0.945	0.862
	100	0.886	0.452	0.229	0.522
Glutathion		1.111	0.734	0.671	
Glutathion $mg.L^{-1}$					
Glutathion x Prolin		0	100	200	Prolin
Prolin $mg.L^{-1}$	0	1.099	0.987	0.669	0.918
	200	0.842	0.854	0.742	0.813
	400	0.667	0.763	0.649	0.693
Ascorbic $mg.L^{-1}$					
Glutathion x Ascorbic		0	50	100	Ascorbic
Glutathion $mg.L^{-1}$	0	0.982	0.824	0.964	0.923
	100	0.938	0.796	0.912	0.882
	200	0.713	0.634	0.510	0.619
L.S.D. 0.05					
L.S.D Glutathion=0.2320					
L.S.D Prolin=0.2320					
L.S.D Ascorbic =0.2320					
L.S.D Glutathion x Prolin =0.4019					
L.S.D Glutathion x Ascorbic =0.4019					
L.S.D Prolin x Ascorbic =0.4019					
L.S.D Glutathion x Prolin x Ascorbic =0.6961					

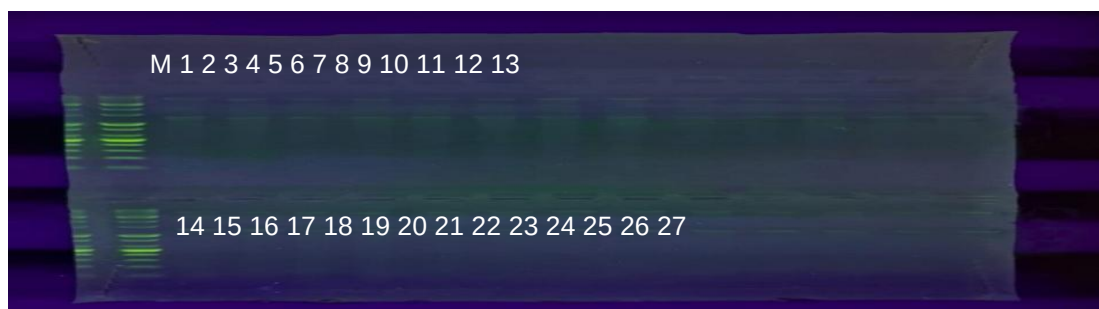
3.2. Molecular Study

3.2.1. Relative Quantification and Gene Expression Analysis of the Glutathione Peroxidase (FaGPX) Gene in the Leaves of Ruby Gem Strawberry Seedlings

3.2.1.1. Extraction of Total RNA from Study Treatment Samples

High-efficiency RNA extraction is important, as it is the first step in producing highly efficient cDNA, the raw material for cDNA synthesis. RNA extraction is a delicate process that is sensitive to extraction conditions. RNA is also sensitive to the chemicals used and requires compatibility between the extraction materials and conditions. The use of a specialized laboratory kit for isolating total RNA from plants is an accurate method, as high efficiency and ease were

achieved by isolating total RNA from the leaves of Ruby Gem Strawberry seedlings. This compatibility is represented by the steps used in the extraction process, the chemicals used, and the use of the filter method. Liquid nitrogen was used, as recommended in the leaflet method, which breaks down the cell walls of plant cells and stops the activity of nuclear enzymes due to the low temperature (96°C), as these enzymes are released once the cell wall is destroyed. The use of DNase I in the total RNA extraction method eliminated gDNA, which would likely pass through the filters, to obtain pure RNA in the gel form. The extraction efficiency is also due to the filters, which isolate and remove unwanted cell components while simultaneously preserving RNA, which is the target of extraction [18].



Figurer 1. Extraction of total RNA from study treatment samples. M = Marker Leader : 1 = P0 G0 A200 , 2 = P400 G0 A0 , 3 = P0 G50 A200 , 4 = P400 G50 A100 , 5 = P0 G100 A200 , 6= P400 G0 A100 , 7 = P0 G100 A0 , 8 = P200 G100 A200 , 9 = P400 G0 A200,10 = P0 G0 A100 ,11= P200 G0 A0 ,12= P0 G50 A0 ,13= P200 G50 A100 ,14= P0 G0A0 ,15= P0 G100 A100 ,16= P200 G50 A200 ,17= P200 G0 A100 ,18= P200 G100 A100 ,19= P400 G50 A0 ,20= P200 G0 A200 ,21= P400 G50 A200 ,22= P0 G50 A100 ,23= P200 G100 A0 ,24= P400 G100 A0 ,25= P200 G50 A0 ,26= P400 G100 A100 ,27= P400 G100 A200.

3.2.1.2. Synthesis of cDNA from RNA Samples Extracted from Experimental Treatments

The success of the cDNA manufacturing process from RNA extracted from the leaves of the Ruby Gem variety of the studied Strawberry was confirmed, as Figure (2) shows the migration sections on agarose gel (1%), indicating the success of the cDNA manufacturing process, as the sections of the bands were bright, clear and distinct.

cDNA synthesis is an important step, as it is the basic material for Real-time PCR. The efficiency and success of the cDNA synthesis process are attributed to the various kits used in the synthesis process, as they contain specialized materials for cDNA synthesis, in addition to the purity of the total RNA extracted from the treated chive leaves and the optimal conditions provided for the Reverse Transcriptase enzyme using a Water Bath device, which operates under high control and with a constant timer and temperature as required. RT-PCR is a modern and ideal technology adopted in cDNA synthesis, especially for detecting gene expression.

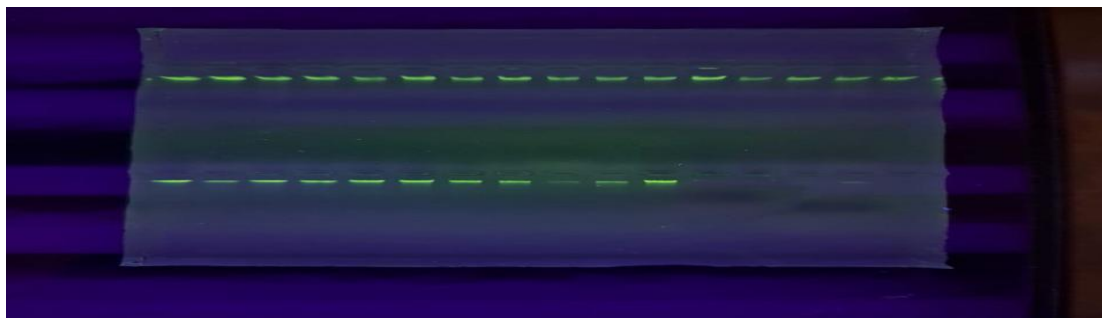


Figure 2. Gel electrophoresis upper synthesis of cDNA from RNA samples extracted from experimental treatments.

3.2.2. Measurement of the Relative Amount of Gene Expression for the Glutathione Peroxidase Gene *FaGPX*

As shown in Figure (3), the Ruby Gem seedlings irrigated with salty well water had the highest gene expression of the glutathione peroxidase gene *FaGPX* in the control treatment (9.849 fold), while the highest gene expression was obtained in the treatment of spraying with ascorbic acid at a concentration of (100 mg L^{-1}) (9.18 fold). The results of the same figure (3) showed that the results were close and high with the spray treatments, while the concentration (200 mg L^{-1}) when spraying with nano-ascorbic acid showed an increase in gene expression of (7.464 fold).

It is evident from the analysis of the relative amount of glutathione peroxidase gene *FaGPX* in the Ruby C variety of strawberry seedlings under the influence of salt stress if the individual treatment of spraying with nano-amino acid proline was given, an increase in gene expression was recorded at a concentration of (200 mg L^{-1}) individually, while spraying with the antioxidant nano-glutathione individually at a concentration of (200 mg L^{-1}) gave the same increase in the percentage of gene expression, which reached (6.964 fold).

Spraying with the antioxidant nano-glutathione alone, at a concentration of (100 mg L^{-1}), resulted in a slight increase compared to the other treatments, reaching (5.278 fold), while the rest of the treatments had intermediate gene expression levels between the highest and lowest values. When spraying the Ruby Gem plants irrigated with saline well water and exposed to salt stresses with compounds that reduce these stresses, as observed from the results of the interactions given by the spraying treatments, the triple interaction treatment between (nano proline, nano ascorbic acid, and nano antioxidant glutathione, with a concentration of ($200 \text{ mg L}^{-1} + 100 \text{ mg L}^{-1} + 200 \text{ mg L}^{-1}$) resulted in the lowest gene expression (0.133 fold). The triple interaction treatment (nano proline, nano ascorbic acid, and nano antioxidant glutathione with a concentration of ($200 \text{ mg L}^{-1} + 100 \text{ mg L}^{-1} + 200 \text{ mg L}^{-1}$) gave the same percentage of gene expression, reaching (0.133 fold), which is the lowest gene expression among the single treatments and the interaction.

As shown in Figure (3), the triple interaction that gave the lowest gene expression for the treatment (nano proline 400 mg L^{-1} , nano ascorbic acid 50 mg L^{-1} , and spraying with the antioxidant nano glutathione 100 mg L^{-1}) reached (0.143 fold), while the rest of the treatments ranged in the level of gene expression between the highest value and the lowest value.

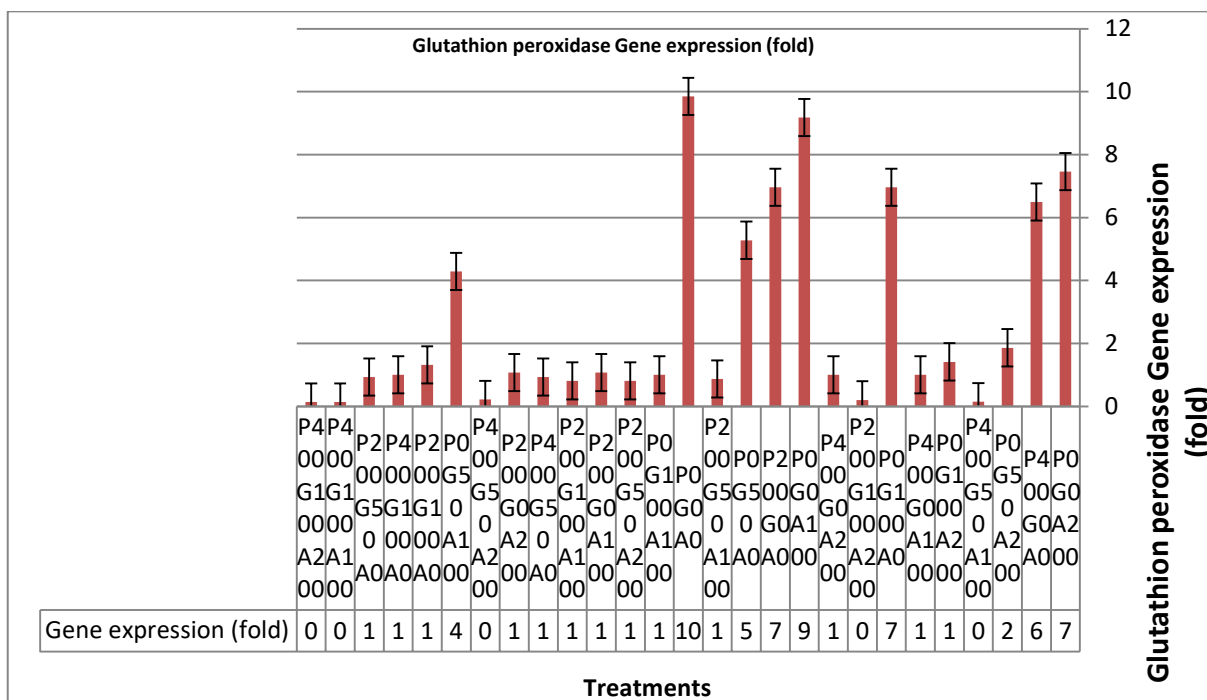


Figure 3. The relative amount of gene expression for the glutathione peroxidase gene FaGPX.

4. Discussion

Proline is found in the protein structure and its inclusion in proteins is controlled by factors affecting protein structure, such as t-RNA modification, the ribosomal translation system, and the increase of ribosomes, in addition to factors controlling the connection of m-RNA to ribosomes. Increasing the activity of the nitrate reductase enzyme, increasing the percentage of ribosomes, and forming the nitrate reductase complex is important in the process of incorporating proline into the protein [19].

Glutathione removes reactive free radical species (ROS), participates in the growth stage and DNA synthesis of the cell, and affects the embryonic stage of root development [20]. Glutathione works to accumulate anthocyanins, and it also represents an important factor in the process of programmed cell death and disease resistance [21], and has an effect on cell differentiation [22]. Glutathione removes stress by binding glutathione with molecules, and then enzymes bind to the outer surface of glutathione [23].

Ascorbic acid plays an important role in plants, as it activates and regulates photosynthesis, supports iron oxidation and reduction, and is involved in the transfer of hydrogen ions from NADH to oxygen. It is also an electron donor and acceptor in plasma membranes and chloroplasts and affects the oxidation, construction, and activity of enzymes. In addition, it plays an important role as an antioxidant in many biological systems, mainly in the photosynthetic system, including chlorophyll, which delays leaf aging and protects the cell from damage (ROS), thus delaying or preventing programmed cell death. It also removes cell toxins by reducing free radicals from their sources. Other vital activities in which the role of ascorbic acid is prominent include cell division and expansion, which increase the effectiveness of cell division when the cell transforms from the vegetative phase to the reproductive phase, as well as its role in metabolic activities at the beginning of germination [24,25].

Many genes work together to control complex traits of salinity tolerance. These genes, including

NHX1, DREB, ABRE, CDPK, and osmotin or osmotin-like protein (OSM1), are members of various families and play a significant role in determining gene expression following stress exposure in relation to salinity tolerance during plant growth, development, and expansion [26,27]. Peroxidase is an essential enzyme in biological systems of aerobic organisms. It is an oxidation-reduction enzyme that catalyzes the conversion of hydrogen peroxide to water, which releases the detrimental effects of hydrogen peroxide from the cells of the organism. As it is a generator of free radicals, hydrogen peroxide may destroy essential substances inside cells and interfere with many of their key functions. Certain plant species are excellent sources of peroxidase because of their high activity [28]. APX genes in plants have been shown to vary in growth and antioxidant metabolism, and the involvement of these enzymes in normal plant growth has been revealed. The expression of APX genes is strongly regulated by stresses including drought and salinity, as well as during plant development [29]. Genes encoding APXs are particularly important for maintaining ascorbate (AsA) and glutathione (GSH), non-enzymatic antioxidants in the context of cellular redox homeostasis and redox signaling, and are directly or indirectly involved in maintaining photosynthesis in plants under stressful environmental conditions [30,31].

Conclusions

The study results showed that spraying with Nano-proline led to significant superiority at a concentration of 0 mg.L^{-1} , as it gave positive results in the activity of the peroxidase enzyme and the activity of the ascorbic peroxidase enzyme. The antioxidant glutathione had a significant effect at a concentration of 200 mg/L^{-1} on the activity of the peroxidase enzyme at a concentration of 200 mg/L . The results showed that spraying with ascorbic acid at a concentration of 100 mg/L^{-1} resulted in a significant increase in peroxidase enzyme characteristics. The interaction of three experimental factors (nanofolate proline 400 mg L^{-1} , nano glutathione 200 mg L^{-1} and nano ascorbic acid 100 mg L^{-1}) had a positive effect on the chemical and molecular indicators of the Ruby Gem plant.

The technique of measuring the relative amount of FaGPX gene expression on the Ruby Gem variety of strawberry is very useful in determining the effect of using the experiment to reduce the effect of salinity on irrigation with well water.

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Impact of Organic Fertilization with Orange Peels and Biofertilization with Mycorrhizal Fungi on *Sorghum bicolor* L. Oil Yield and Chemical Characteristics

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Abstract. The aim of the study is to use a biological and organic fertilization alternative to chemical fertilization, which harms human health, in order to improve the oil yield and chemical properties (nitrogen, phosphorus, potassium, proline, and zinc) of *Sorghum bicolor* L. For the 2023 growing season, a mixed soil field experiment was conducted in one of the fields in Al-Muhannawiya Village, Al-Sada District, Babil, Iraq. Seeds were then planted on March 19, 2024. Two factors were included in the experiment, which was carried out using a factorial arrangement and a randomized complete block design (RCBD) with three replications: mycorrhizal fungi were injected into the rhizosphere, or the area close to the roots (control, 50, 75, and 100 ml/L) 30 days after planting, and the second factor was the concentration of orange peels mixed with soil (control, 50, 75, and 100 g/mL). Two dosages of the medications were administered, and the plants were harvested when they were fully grown after all growth indicators were tracked. Based on the results, which indicate that the effects of mycorrhizal ml L⁻¹ and orange peel treatments at a concentration of 100 gm L⁻¹, there were significant variations in the oil yield percentage (45.28, 45.50 %), protein (13.09, 12.645%), zinc (35.93, 36.52%), nitrogen (0.9415, 0.975%), boron (31.23, 32.22%), potassium (9.94, 10.28%), phosphorus (0.3048, 0.2981%), proline (10.60, 10.94 mg/g) respectively.

Keywords. Mycorrhizal Fungi, *Sorghum bicolor* L. Orange Peels .organic fertilization.

1. Introduction

The agricultural sector in many Lower to lower middle income countries suffers from excessive reliance on chemical fertilizers, which leads to degradation of soil fertility, increased environmental pollution, and higher agricultural production costs. In light of the global shift towards sustainable agriculture, there is a growing need to adopt alternative plant nutrition methods that are more efficient and less harmful to the environment. Despite studies indicating the effectiveness of both bio-fertilization and organic fertilization individually, the interaction

between mycorrhizal fungi and organic agricultural waste, such as orange peels, has not been sufficiently studied in white corn, leaving a research gap that warrants exploration.

White sorghum (*Sorghum bicolor* L.) is considered It is a strategic crop in many arid and semi-arid regions because of its ability to withstand harsh environmental conditions and its nutritional, industrial, and fodder importance. With the increasing environmental pressures associated with the intensive use of chemical fertilizers, there has been an urgent need for more sustainable and environmentally friendly alternatives, including bio-fertilization and organic fertilization [1].

Among the most prominent types of biofertilizers used recently are mycorrhizal fungi (Arbuscular Mycorrhizal Fungi—AMF), which are considered beneficial microorganisms that live in a symbiotic relationship with plant roots. They contribute to enhancing the absorption of nutrients, especially phosphorus, and improving plant resistance to biotic and abiotic stresses [2]. Mycorrhizae also play an important role in improving the physical and chemical properties of the soil, which positively affects crop growth and productivity, including white corn[3]. The application of mycorrhizal fungi was noteworthy. Particularly in settings with nutrient deficiencies or environmental stress, these helpful fungi coexist symbiotically with the roots of the plant, improving the absorption of water and nutrients. Enhancing nutrient absorption: It improves the absorption of certain trace elements, such as copper, zinc, and phosphorus in particular [4].By extending plant roots through the hyphal network, mycorrhizae expand the area that can absorb nutrients. Enhancing plant stress tolerance lessens the effects of environmental stressors such as salinity, drought, and poor soil. aids in controlling the soil's absorption of water [5].

On the other hand, research is moving towards using alternative organic sources rich in beneficial compounds, such as orange peels, which are recyclable agricultural waste with a high content of carbohydrates, fiber, and volatile oils, making them an improved organic soil amendment upon decomposition [6]. Studies have indicated that adding these peels may improve microbial activity in the soil, provide important nutrients, and contribute to enhanced plant growth [7]. Flavonoid s, volatile aromatic oils, vitamin C, carotene, and resins are among the numerous active chemical compounds found in orange peels [8]. Additionally, peels contain anti-insect agents, such as parasites, worms, and manure [9].Therefore, this study aimed to investigate the integrated effect of bio-fertilization with mycorrhizal fungi and organic fertilization with orange peels on the growth and productivity of white corn by evaluating the oil yield and chemical characteristics. The results of this study research are expected to contribute to supporting the trend towards sustainable agriculture and enhancing the utilization of local renewable resources.

2. Material and Method

2.1. For the 2023 growing season, a mixed soil field experiment was carried out in one of the fields in Al-Muhannawiya Village, Al-Sada District, Babil, Iraq.

2.2. Orange Extraction

The oranges were purchased from local markets, the orange peels were taken, dried in the shade, and the dried peels were ground using an electric grinder [11].Extracting orange peels using a Soxhlet apparatus: Take 50 grams of ground peels and place them in the thimble. The flask was then placed in a 70% ethanol solution, and the take the alcohol in flask. The Soxhlet apparatus was operated at 70°C for 24 hours. The extract was then taken and placed in a rotary evaporator to separate the alcohol from the extract. Then, take the crude extract and prepare it according to the concentrations indicated in the method above [12].

2.3. Soil Service Operation

The following soil service operations were performed: leveling (using disc harrows), plowing (using a reversible moldboard plow), and then splitting into multiple experimental units. The experimental unit area was 15 square meters, or 5 m × 3 m.. To ensure that there was no effect transfer between them, 75 cm and 1 m gaps were left between the replicates. The seeds were then planted on March 19, 2024, in holes in the upper third of the row, 50 cm apart, alternating on both sides of the row, with three seeds per hole and a depth of three to five centimeters. The plants were then reduced in number to one per hole. All crop maintenance procedures were also completed, including four weedings during the crop growth period and periodic irrigation of the plants according to plant health and soil moisture levels. Additionally, nitrogen fertilizer in the form of urea 200 kg ha⁻¹ (N46%) was applied to the soil in two doses, one month after planting and the second before the flowering stage, in accordance with the fertilization recommendations [9]. Diammonium Phosphate (DAP), a phosphate fertilizer, was 160 kg ha⁻¹. Two factors were included in the experiment, which was carried out using a factorial arrangement and a randomized complete block design (RCBD) with three replications: mycorrhizal fungi were injected into the rhizosphere, or the area close to the roots (control, 50, 75, and 100 ml/L) 30 days after planting, and the second factor was the concentration of orange peels mixed with soil (control, 50, 75, and 100 g/mL). Two dosages of the medications were administered, and the plants were harvested when they were fully grown after all growth indicators were tracked [10].

2.4. The oil yield and percentage of 50 g of fin powder were extracted for 24 hours with 150 ml of 70% hexane in a 500 ml round-bottom flask using a Soxhlet apparatus in a thimble, and the extract was then evaporated using a spinning evaporation apparatus at 45°C [13]

2.5. Measuring the Percentages of Nutrients

Collected the leaves from under the main artichoke leaf, from five plants per experimental unit, and dried them in an electric oven at 65°C for 72 hours until the weight stabilized. I then took samples of the grains and ground them. 0.2 grams of the ground samples (0.2 g) were collected, and the samples were digested by adding 4 ml of concentrated sulfuric acid and 2 ml of concentrated perchloric acid. These The mentioned elements have been estimated [14].

Nitrogen and protein levels were determined using the Kjeldahl apparatus and according to a previously described method [14].

Phosphorus was determined using ascorbic acid and ammonium molybdate with a spectrophotometer at a wavelength of 620 nm [14].

Potassium was measured using a flame photometer [14], and the zinc percentage An adsorbent metric spectrophotometer was used to measure the amount of zinc in 2.0 g of ground seeds after they had been digested in a solution of sulfuric and hydrochloric acids. Atomic ions were measured using a particular technique and a spectrophotometer [14].

Proline using the ninhydrin reagent according to the described method [15].

Poron The boron concentration was measured using a spectrophotometer, according to the method described in [16].

2.6. Statistical Analysis

The experimental data were statistically analyzed using variance analysis according to the Randomized Complete Block Design (RCBD) for factorial experiments. The significant differences between the means of the various treatments were compared using the Least Significant Difference (L.S.D.) test at a 5% probability level, using the statistical software (GenStat 2018) for data analysis [17].

3. Results

The results in Table (1) clearly show that the effects of mycorrhizal mL/L treatments result in significant variations in the percentage of oil content of seeds. With the highest average concentration of 45.28%, the concentration of 100 mL/L⁻¹ was followed by the 75 and 50 mL/L⁻¹ treatments, which had average concentrations of 44.55 and 42.98 percent, respectively. In contrast On the other hand, the control average protein content of the treatment plants was the lowest, at 41.13%. The same table shows the orange peel fertilizer treatments performed significantly better, achieving concentrations of 100 mL/L⁻¹, which resulted in the highest average protein content, 45.50%. By In contrast, the treatment control group had the lowest average (41.195%). Then came the 75 and 50 mL/L-1 treatments, which had respective average concentrations of 44.41% and 42.93%, respectively.

Concerning how these two factors (mycorrhizal mL/L and orange peel mL/L fertilizer at 50 mL/L concentrations) interact, a noteworthy interplay is apparent in the percentage of oil. In contrast to the plants treated with the dual-interaction control, which displayed the lowest average of 39.65%, the plants treated with the dual-interaction (100 mL/L⁻¹) displayed the highest average of 47.84%.

Table 1. Effect of the mycorrhizal and orange peel on the percentage of oil %.

Orange peel mL ⁻¹	Mycorrhizal mL ⁻¹				Mean
	Control	50	75	100	
Control	39.65	40.63	41.74	42.76	41.195
50	40.56	42.76	43.84	44.56	42.93
75	41.56	43.84	45.86	46.36	44.405
100	42.76	44.67	46.74	47.84	45.50
Mean	41.1325	42.975	44.545	45.28	
LSD _{0.05} Orange peel : 1.58					
Mycorrhizal:1.64					
Interaction: 2.65					

Based on the results of Table (2), it clearly shows that the effects of mycorrhizal mL/L treatments result in significant variations in the protein content of seeds. With the highest average concentration of 13.09%, the concentration of 100 mL/L⁻¹ was followed by the 75 and 50 mL/L⁻¹ treatments, which had average concentrations of 12.2275 and 10.5725 percent, respectively. In contrast On the other hand, the average protein content of the treatment plants was the lowest, at 9.6025%. As can be seen from the same table, the orange peel fertilizer treatments performed significantly better, achieving concentrations of 100 mL · L⁻¹, which resulted in the highest average protein content of 12.645%. In contrast, the treatment control group had the lowest average (10.0075%). Then came the 75 and 50 mL/L⁻¹ treatments, which resulted in respective average concentrations of 11.6925 and 11.1725%, respectively. Regarding the interplay between these two variables (mycorrhizal mL/L and orange peel mL/L fertilizer at 50 mL/L concentrations), a noteworthy interplay was apparent in the protein percentage. In contrast to the plants treated with the dual-interaction control, which displayed the lowest average of 8.56%, the plants treated with the dual-interaction (100 mL/L⁻¹) displayed the highest average of 14.84%.

Table 2. Impact of orange peel and mycorrhizae on the proportion of protein in seeds.

Orange peel mL ⁻¹	Mycorrhizal mL ⁻¹				Mean
	Control	50	75	100	
Control	8.56	9.45	10.46	11.56	10.0075
50	9.45	10.46	11.94	12.84	11.1725
75	9.95	10.93	12.67	13.12	11.6925
100	10.45	11.45	13.84	14.84	12.645

Mean	9.6025	10.5725	12.2275	13.09
	LSD _{0.05} Orange peel 1.73:			
	Mycorrhizal: 1.37			
	Interaction: 2.65			

The findings in Table (3) unequivocally demonstrate that the impacts of mycorrhizal mL/L treatments lead to notable fluctuations in the zinc percentage. The 75 and 50 mL/L-1 treatments had average concentrations of 34.88 and 32.72 percent, respectively, while the concentration of 100 mL/L-1 had the highest average concentration of 35.93 percent. In contrast On the other hand, the treatment plants' control average zinc content of the treatment plants was the lowest, at 32.72%. However, the orange peel fertilizer treatments performed significantly better, achieving concentrations of 100 mL · L⁻¹, which resulted in the highest average percentage of zinc content (36.52%). In contrast, the treatment control group had the lowest average (32.18%). Then came the 75 and 50 mL/L-1 treatments, which had respective average concentrations of 35.32% and 33.21%. Indicate the interaction between these two variables (mycorrhizal mL/L and orange peel mL/L fertilizer at 50 mL/L concentrations); a noteworthy interplay is apparent in the percentage of zinc. In contrast to the plants treated with the dual-interaction control, which displayed the lowest average of 30.65%, the plants treated with the dual-interaction (100 mL/L⁻¹) displayed the highest average of 38.75%.

Table 3. Effect of the mycorrhizal and orange peel on the percentage of zinc.

Orange peel mL ⁻¹	Mycorrhizal mL ⁻¹				Mean
	Control	50	75	100	
Control	30.65	31.56	32.95	33.56	32.18
50	31.65	32.75	33.87	34.56	33.21
75	33.75	34.84	35.84	36.84	35.32
100	34.74	35.74	36.84	38.75	36.52
Mean	32.7225	32.72	34.875	35.93	
LSD _{0.05} Orange peel=1.65 ;, Mycorrhizal= 1.23 , Interaction=2.54					

Based on the results of Table (4), it is clear that the effects of mycorrhizal mL/L treatments result in significant variations in the percentage of boron. With the highest average concentration of 31.23%, the concentration of 100 mL/L⁻¹ was followed by the 75 and 50 mL/L⁻¹ treatments, which had average concentrations of 29.08 and 27.15 percent, respectively. On the other hand, the control average percentage of boron content in the treatment plants was the lowest, at 23.27%. From the same table, it can be seen that the orange peel fertilizer treatments performed significantly better, achieving concentrations of 100 mL · L⁻¹, which resulted in the highest average percentage of boron content (32.22%). By In contrast, the treatment control group had the lowest average of 22.91%. Then came the 75 and 50 mL/L⁻¹ treatments, which had respective average concentrations of 29.11 and 26.65%. However, in the interaction between these two variables (mycorrhizal mL/L and orange peel mL/L fertilizer at 50 mL/L concentrations), a noteworthy interplay is apparent in the percentage of boron. In contrast to the plants treated with the dual-interaction control, which displayed the lowest average of 36.53%, the plants treated with the dual-interaction (100 mL/L⁻¹) displayed the highest average of 19.65%.

Table 4. Effect of the mycorrhizal and orange peel on the percentage of boron (%).

Orange peel mL ⁻¹	Mycorrhizal mL ⁻¹				Mean
	Control	50	75	100	
Control	19.65	21.74	24.47	25.76	22.91
50	22.65	26.56	27.56	29.74	26.65

75	24.67	28.45	30.45	32.87	29.11
100	26.75	31.74	33.84	36.53	32.22
Mean	23.27	27.15	29.08	31.23	

LSD_{0.05} Orange peel 1.72; Mycorrhizal:1.65 , Interaction: 2.34

Indicate that the results in Table (5) clearly show that the effects of mycorrhizal mL/L treatments result in significant variations in the percentage of potassium (%). With the highest average concentration of 9.94%, the concentration of 100 ml/L-1 was followed by the 75 and 50 ml/L-1 treatments, which had average concentrations of 8.58 and 7.08 percent, respectively. In contrast On the other hand, the control average percentage of potassium content in the control control-treatment plants was the lowest, at 5.43%. The same table shows the orange peel fertilizer treatments performed significantly better, achieving concentrations of 100 ml/L⁻¹, which resulted in the highest average percentage of potassium content, 10.28%. In contrast, the treatment control group had the lowest average (5.29%). Then came the 75 and 50 ml/L-1 treatments, which had respective average concentrations of 8.43 and 7.03%, respectively. In the interaction between these two factors (50 ml/L⁻¹ concentrations of mycorrhizal ml/L and orange peel ml/L fertilizer), a noteworthy interplay is apparent in the percentage of potassium. In contrast to the plants treated with the dual-interaction control, which displayed the lowest average of 3.76%, the plants treated with the dual-interaction (100 ml/L⁻¹) displayed the highest average of 12.65%.

Table 5. Effect of the mycorrhizal and orange peel on the percentage of potassium (%).

Orange peel mL ⁻¹	Mycorrhizal mL ⁻¹				Mean
	Control	50	75	100	
Control	3.76	4.76	5.78	6.86	5.29
50	4.65	6.45	7.45	9.56	7.0275
75	5.76	7.45	9.84	10.67	8.43
100	7.56	9.67	11.23	12.65	10.28
Mean	5.43	7.08	8.58	9.94	

LSD_{0.05} Orange peel :2.94, Mycorrhizal: 2.16, Interaction: 3.65

Table (6) findings unequivocally demonstrate that the effects of mycorrhizal mL/L treatments cause notable fluctuations in the phosphorus percentage. With the highest average concentration of 0.3048%, the concentration of 100 ml/L⁻¹ was followed by the 75 and 50 ml/L⁻¹ treatments, which had average concentrations of 0.2788 and 0.2345 percent, respectively. On the other hand, the control average percentage of potassium content of the treatment plants was the lowest, at 0.2108%.

However, the orange peel fertilizer treatments performed significantly better, achieving concentrations of 100 ml · L⁻¹, which resulted in the highest average percentage of phosphorus content, 0.2981%. In contrast, the treatment control group had the lowest average (0.2210%). Then came the 75 and 50 ml/L⁻¹ treatments, which resulted in respective average concentrations of 0.2641 and 0.2457%.

This indicates the interplay between these two variables (mycorrhizal ml/L and orange peel ml/L fertilizer at 50 ml/L concentrations), and a noteworthy interplay is apparent in the percentage of phosphorus. In contrast to the plants treated with the dual-interaction control, which displayed the lowest average of 0.1745%, the plants treated with the dual-interaction (100 ml/L⁻¹) displayed the highest average of 0.3476%.

Table 6. Effect of the mycorrhizal and orange peel on the percentage of phosphorus.

Orange peel mlL ⁻¹	Mycorrhizal ml.L ⁻¹				Mean
	Control	50	75	100	
Control	0.1745	0.2045	0.2476	0.2574	0.2210
50	0.1954	0.2165	0.2754	0.2954	0.2457
75	0.2145	0.2486	0.2745	0.3187	0.2641
100	0.2587	0.2685	0.3176	0.3476	0.2981
Mean	0.2108	0.2345	0.2788	0.3048	
LSD _{0.05} Orange peel : 1.35, Mycorrhizal: 1.94, Interaction: 2.53					

Considering the findings in Table (7), clearly show that the effects of mycorrhizal ml/L treatments result in significant variations in the percentage of proline (mg/g). With the highest average concentration of 10.60, the concentration of 100 ml/L-1 was followed by the 75 and 50 ml/L⁻¹ treatments, which had average concentrations of 9.30 and 07.75 mg/g-1 percent, respectively. On the other hand, the control average percentage of proline mg.g⁻¹ content of the treatment plants was the lowest, at 6.51 mg.g⁻¹.

As can be seen from the same table, the orange peel fertilizer treatments performed significantly better, achieving concentrations of 100 ml · L⁻¹, which resulted in the highest average percentage of proline content, 10.94 mg.g⁻¹.. In contrast, the treatment control group had the lowest average of 5.97 mg/g. Then came the 75 and 50 ml/L⁻¹ treatments, which had respective average concentrations of 9.43 and 7.83 mg/g.

Regarding the interplay between these two variables (mycorrhizal ml/L and orange peel ml/L fertilizer at 50 ml/L concentrations), a noteworthy interplay was apparent in the percentage of proline (mg/g). In contrast to the plants treated with the dual interaction control, which displayed the lowest average of 4.65 mg.g⁻¹, the plants treated with the dual interaction (100 ml·L⁻¹) displayed the highest average of 13.86 mg.g⁻¹.

Table 7. Illustrates the impact of mycorrhizal and orange peel on the proline percentage (mg/g).

Orange peel mlL ⁻¹	Mycorrhizal ml.L ⁻¹				Mean
	Control	50	75	100	
Control	4.65	5.55	6.34	7.34	5.97
50	5.96	7.45	8.45	9.45	7.83
75	6.76	8.56	10.65	11.76	9.43
100	8.67	9.45	11.76	13.86	10.94
Mean	6.51	7.75	9.30	10.60	
LSD _{0.05} Orange peel 1.95; Mycorrhizal: 1.38, Interaction: 2.34					

Indicate T The results in Table (8) clearly show that the effects of mycorrhizal mL/L treatments result in significant variations in the percentage of nitrogen. With the highest average concentration of 0.9415%, the concentration of 100 ml/L⁻¹ was followed by the 75 and 50 ml/L⁻¹ treatments, which had average concentrations of 0.89425% and 0.8285%, respectively. On the other hand, the control average percentage of nitrogen content of the treatment plants was the lowest, at 0.75025%.

As can be seen from the same table, the orange peel fertilizer treatments performed significantly better, achieving concentrations of 100 ml · L⁻¹, which resulted in the highest average percentage of nitrogen content (0.975%). In contrast, the treatment control group had the lowest average score of 0.7003. Then came the 75 and 50 ml/L⁻¹ treatments, which resulted in average concentrations of 0.946 and 0.79325%, respectively.

indicate the interplay between these two variables (mycorrhizal ml/L and orange peel ml/L fertilizer at 50 ml/L concentrations), a noteworthy interplay is apparent in the percentage of nitrogen. In contrast to the plants treated with the dual-interaction control, which displayed the lowest average of 0.546%, the plants treated with the dual-interaction (100 ml/L⁻¹) displayed the highest average of 0.999%.

Table 8. Effect of mycorrhizal and orange peel on percentage of nitrogen (%).

Orange peel ml\L ⁻¹	Mycorrhizal ml.L ⁻¹				Mean
	Control	50	75	100	
Control	0.546	0.654	0.745	0.856	0.7003
50	0.654	0.748	0.856	0.915	0.79325
75	0.856	0.945	0.987	0.996	0.946
100	0.945	0.967	0.989	0.999	0.975
Mean	0.75025	0.8285	0.89425	0.9415	
LSD _{0.05} Orange peel :1.84, Mycorrhizal: 1.47, Interaction: 2.43					

4. Discussion

The results of this study indicate that combining biofertilization with mycorrhizal fungi and organic fertilization with orange peels not only improves the growth and productivity of white corn but also contributes to enhancing the quality of oil yield and chemical characteristics. This improvement is particularly significant in the context of public health, as these compounds have demonstrated proven antioxidant, anti-inflammatory, and protective properties against certain chronic diseases such as heart disease and cancer [18].

This improvement was attributed to a complex set of biological interactions occurring at the soil, root, and plant levels. Mycorrhizal fungi enhance the absorption of micronutrients such as zinc, iron, and magnesium, which play important roles as enzyme cofactors in the biosynthetic pathways of phenolic compounds and antioxidants [19]. Previous studies have confirmed that plants associated with mycorrhizae exhibit higher activity of enzymes related to plant defense, which increases the synthesis of volatile oil [20].

With regard to the treatment of orange peels at the above concentrations, there has been a moral increase in the concentration of oil, carbohydrates, protein, and nitrogen when orange peels are treated at the above concentrations, possibly due to the fact that they contain a number of active chemical compounds, especially flavonides, carotene, vitamins E and C, and steric acid [21]. These compounds also act as antioxidants, which protect plant cells from harmful free radicals that attack cells by oxidizing chlorophyll, fat, proteins, cellular membranes, and DNA, which causes the plant to age and die too soon [21]. Oranges shield plant tissues when exposed to environmental conditions that negatively affect plant vegetative and metabolic processes, such as heat, light, cold, and radiation [22]. In contrast On the other hand, orange peels, which are high in simple sugars, organic acids, and volatile oils, provide a rich organic medium that contributes to the nourishment of beneficial microorganisms in the soil and enhances their metabolic activity [23]. This activity leads to the decomposition of organic matter and the release of compounds that are effective in promoting the physiological activity of plants, including stimulating the secondary metabolic pathways associated with the synthesis of flavonoids and carotenoids (24).

The dual-treatment mycorrhiza showed the highest effect, with the grains recording the highest concentration of active compounds, in addition to a significant increase in the content and quality of the extracted oils in terms of fatty acid composition, particularly an increase in the percentage of unsaturated fatty acids, which reflects an improvement in the health value of the resulting oil [25].

These results support the hypothesis that bio-fertilization and organic fertilization not only affect growth and productivity but also extend to improving the quality of agricultural products in terms of functional content and compounds with positive effects on human health. Therefore, this makes these techniques effective tools for producing crops with high nutritional value within sustainable agriculture systems [26].

Conclusion

White sorghum showed a remarkable positive response to bio-agricultural and organic improvements, making it suitable as a functional food crop with health benefits that can be developed through sustainable agriculture programs and the production of foods with protective effects on human health.

fertilization-Bio and organic fertilization represent promising alternatives to chemical fertilizers in producing crops with high nutritional quality and reflect a practical trend towards achieving food and nutritional security through environmentally friendly means.

Mycorrhizal ml L⁻¹ and orange peel treatments and their interaction at a concentration of 100 gm L⁻¹: there were significant variations in the oil yield percentage, protein, zinc, nitrogen, boron, potassium, phosphorus, and proline.

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Phytoremediation of Some Heavy Metals from Aqueous Solutions Using *Hydrilla verticillata* and *Lemna minor*

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Abstract. Heavy metals pose a global threat to aquatic ecosystems. This study aimed to evaluate the efficiency of *Hydrilla verticillata* and *Lemna minor* plants in removing Cd, Pb, and Ni from aqueous solutions and to study the effects of these metals on plant physiological characteristics. The phytoremediation technique was applied in a laboratory setting, where plants were exposed to different metal concentrations, and the removal rate, bioconcentration factor (BCF), biomass, and total chlorophyll content were measured. The results showed that *L. minor* excelled at metal removal, achieving the highest removal efficiencies of 99.8% for nickel, 99.7% for lead, and 92% for cadmium. These results were consistent with the very high BCF values, confirming their superior ability to accumulate metals. In contrast, *H. verticillata* showed lower efficiency, with the highest removal rate of 89.19% for nickel (Ni), 76.67% for lead (Pb), and 72.2 for cadmium. The results also showed that both plants experienced physiological stress, with both biomass and total chlorophyll content declining with increasing metal concentrations, particularly in *Hydrilla* plants. This study concludes that *L. minor* represents a promising and effective option for phytoremediation applications in heavy metal-contaminated water, making it a natural biological solution for treating pollutants in Iraq's aquatic environment.

Keyword. Aqueous, Heavy metals, Phytotechnology, Removal ratio, Bioconcentration,

5. Introduction

Phytoremediation is a relatively new term that first appeared in the literature in 1991. "Phytoremediation" comes from the Greek prefix phyto, which means 'plant', and the Latin root remedium, which means 'to repair or remove pollutants' [1].

Phytoremediation is a broad term that refers to techniques that use plants and/or associated microorganisms to reduce, remove, or detoxify pollutants, such as heavy metals, organic pollutants, radionuclides, pesticides, petroleum hydrocarbons, and solvents from soil, water, and air [2].

The choice of plant species used as phytoremediators depends on several factors, including their abundance, growth rate, tolerance, and capacity to accumulate and/or degrade pollutants [3].

They offer many advantages over conventional methods of remediating contaminated land, such as lower cost, maintenance, worker exposure, sustainability, and aesthetic appeal of the

remediation process. They can be applied to a wide range of pollutants and used in conjunction with other remediation techniques. However, they have limitations, such as relatively slow remediation rates, dependence on specific plant species, and the need for long-term monitoring [4,5,6].

One of the local studies conducted on plant treatment was conducted by Mahdi [7], who investigated the efficiency of phytoremediation of wastewater using two submerged aquatic plants (*Ceratophyllum demersum* and *Najas marina*) for wastewater flowing from the secondary sedimentation tank of Hamdan station in Basra City. The results showed that *C. demersum* was more efficient in accumulating and removing the two elements, whereas *N. marina* was more effective in removing nickel than lead.

In another study, Al-Zurfi and Al-Tabtabaei [8] dealt with the ability of *Hydrilla verticillata* to remove Cd and Pb from polluted water, and effective results appeared on the Cd and Pb elements greater than their absorption off Cd, as is actually the case with the effective concentration of the metal, and it began to invent the ability to invent Cd and Pb in the cell, and the high vinyl concentration of the Cd element caused the appearance of the red color in the external appearance of the plant leaves.

Al-Abbawy et al. [9] indicated the efficiency of six species of aquatic plants *Azolla filiculoides*, *Ceratophyllum demersum*, *Potamogeton pectinatus*, *Najas marina*, *Phragmites australis*, and *Typha domingensis* in treating water contaminated with heavy elements such as (iron, nickel, chromium, cadmium, lead, and zinc) in Al-Hawizeh Marsh - southern Iraq. The results showed that the concentrations of Cd, Cr, and Fe in aquatic plants were higher than the World Health Organization (WHO) standards. The concentrations of Zn, Cu, and Pb were within the permissible limits. The results also demonstrated the efficiency of *C. demersum* and *N. marina* in removing and accumulating heavy metals compared to other aquatic plants.

Ali et al. [5] explained the necessity of using aquatic plants to treat wastewater due to their ability to absorb and accumulate pollutants within their tissues, including hydrocarbons and other dangerous and carcinogenic compounds. Aquatic plants have been used in the bioremediation process, as demonstrated in previous studies [10].

6. Material and Methods

This study was conducted between January and November 2024. The collected aquatic plants were as follows: *Hydrilla verticillata* (Figure 3-2) from Al-Abbasiyah Shatt (center Abbasiyah city), which is located at Longitude °32 4' 11.718''N and Latitude °44 26' 47.927''E *Lemna minor* (Figure 1) from Hawizeh Marsh (Um-Al-Naej pond), located at Longitude °47 35 17.657''N Latitude °31.35 13.455 (Figure 1). They plants were brought to the laboratory in polyethylene bags and washed well with river water. The samples were then washed several times with tap water and distilled water to clean them of dirt and materials that were attached and to remove the remnants of adherent plankton and small river animals. After washing the plants, ultrasonic waves were used to isolate the algal cells. The plants were then planted for 14 days to adapt to living in tap water enclosures. Terminal shoots of *H. verticillata* were cut into 10 cm (40 individual) sections, and the branches (flower buds and roots) were removed, while 30 g of *Lemna minor* were cultivated in monocultures within plastic enclosures measuring 30 cm in length, 20 cm in width, and 18 cm in height, each containing 8 liters of tap water. The water level in each container was consistently regulated to account for evaporation during the experiment. The aquatic plants were identified using references specific to Iraq [11, 12]. The experiment for each plant was conducted in 30 plastic enclosures, allocated as follows: nine enclosures for Cd treatments, nine enclosures for Pb treatments, nine enclosures for Ni treatments, and three enclosures as a control. Additionally, three replicates were included for each element

concentration, comprising three treatments exposed to varying Cd concentrations (0.5, 2, and 4 ppm), three treatments exposed to differing Pb concentrations (0.5, 5, and 10 ppm), and three treatments exposed to different Ni concentrations (1, 5, and 10 ppm) (Figure 3-4, Table 3-1). The experiment was conducted using a completely randomized design, and plant parameters (chlorophyll content, protein content, catalase, superoxide dismutase, MDA, plant length, and biomass) were measured after 1, 7, 14, and 21 days of cultivation (Figure 3-5) under constant light (1500 lx), a photoperiod of 16/8 h light/dark, and a temperature of 28 °C.

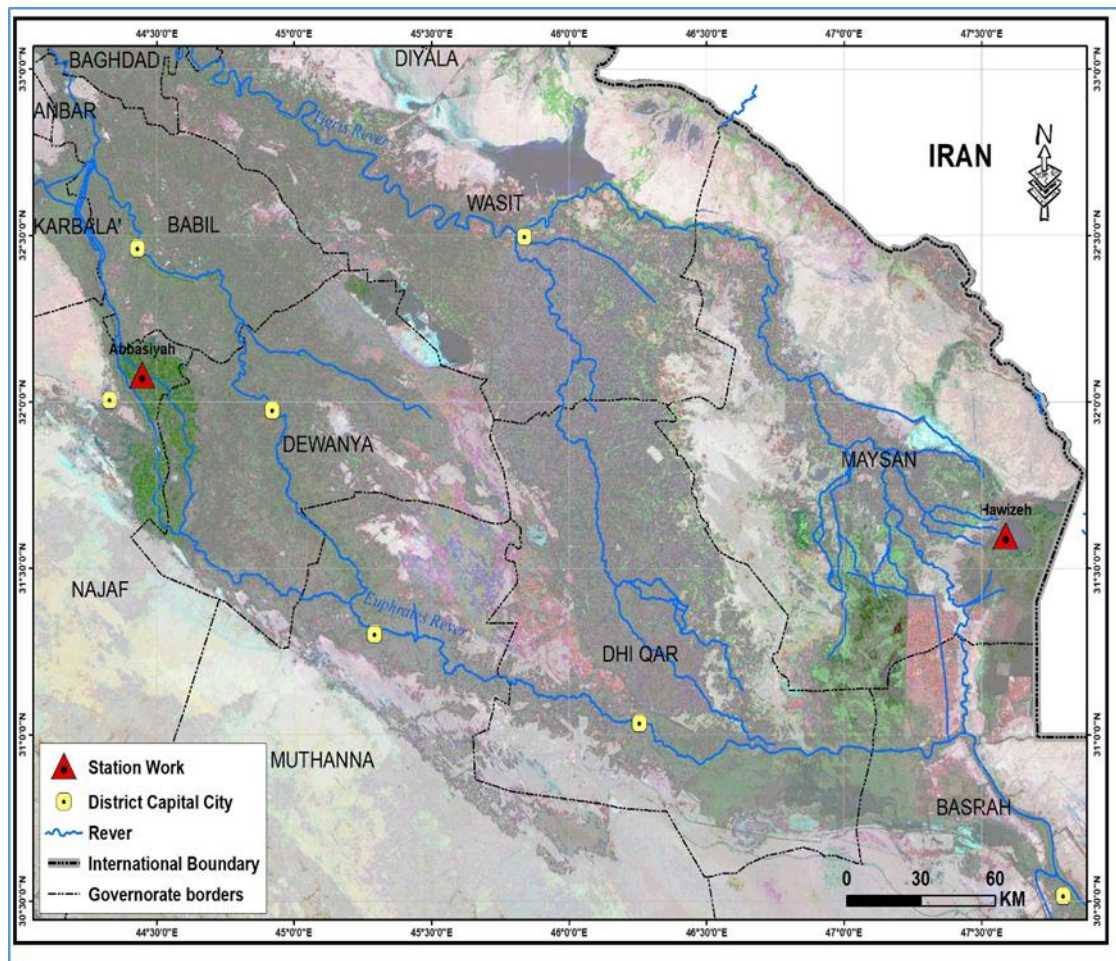


Figure1. A Map of study sampling collection sites (MWR ,2023).

6.1. Plant Measurements and Physiological Methods

6.1.1. Chlorophyll Content

Fresh plant material (0.15 g) was cut into small pieces and placed in a tube for extraction. Then, 2 mL of 80% aqueous acetone was added. The samples were then ground meticulously with a pestle on ice for 30 s (or until a fine slurry was obtained). Next, the pestle was rinsed with the extraction solution, and 3 ml of solvent was added to the tube. To ensure complete chlorophyll extraction, the tubes were placed on ice or in a dark refrigerator for two hours. The extract was then transferred to a centrifuge tube. Then, 5 ml of 80% aqueous acetone was added. The sample was then centrifuged for 20 min at approximately $500 \times g$. Aqueous acetone (80 %) was used as a

blank to calibrate the spectrophotometer. Samples were measured for absorbance at 645 and 663 nm[13].

were calculated using the following formulae [14]:

Total chlorophyll (mg/g)=[A645(20.2)+A663(8.02)] $\times$ v/w $\times$ 1000

Chl.a (mg/g)=[12.7(A663)-2.69 (A645)] $\times$ v/w $\times$ 1000

Chl.b (mg/g)=[22.9(A645)-4.68 (A663)] $\times$ v/w $\times$ 1000

Total carotene (mg/g)=10⁻⁴ (A470)/2592 $\times$ W

A645=Optical absorbtion a wave length 645

A663= Optical absorbtion a wave length 663

A470= Optical absorbtion a wave length 470

W=weight tissue, V=Final volume

6.1.2. Heavy Metal

6.1.3. Digested plant to measure heavy metals

6.1.4. BCF

Plant samples were meticulously cleaned with distilled water, dried, crushed, and sieved through a 63 μ m mesh. A 1 g aliquot was placed into a Teflon beaker with 5 ml of nitric acid and 2 ml of perchloric acid and heated at 70°C until complete dissolution of the materials occurred. Consequently, a 30-minute escalation at a temperature of 200 °C is required to achieve a clear solution [15].

To determine the disposal of the components in the solution, the sample was collected during the 0.45 μ m filtration, subsequently washed with water and acid, and pre-dried [16]. The samples were diluted with deionized water to a final volume of 50 ml and filtered. Samples were stored in hermetically sealed HDPE vials for later analysis using an Atomic Absorption Spectrophotometer [17]. Subsequently, the bio-concentration factor (BCF) of heavy metals was calculated using formula 1.

$$BCF=CB/CWD \quad (1)$$

CB=Chemical Concentration in plant mg/kg⁻¹

CWD= Freely dissolved chemical concentrations in the water mg.l⁻¹

The removal ratio was measured using Formula 2[18].

$$\text{Removal ratio}\% = C1 - \frac{Cf}{C1} \times 100 \quad (2)$$

Where

C1= initial concentration

Cf= Final concentration

6.1.5. Biomass (g/L)

Biomass was determined based on the fresh weight of *H. verticillata* and *L. minor*. After the experiment, the plants were rinsed with tap water and subsequently dried at room temperature for biomass measurement, followed by weighing to obtain a stable weight [19].

Biomass g/L=(initial weight-dry weight)/(volume of enclosure).

6.2. Statistical Analysis

Univariate analysis of variance was used to analyze the data and compare the means of several treatments at $P \leq 0.05$ using the SPSS statistical software (v.26.0). The mean and standard deviation were used for the data. The LSD was used to compare the means of the treatments and days of the experiment and correlation by Pearson correlation. The assumptions underlying these tests (e.g., normal distribution and equal variance) were verified.

7. Results and Discussion

7.1. Removal Ratio

The results showed that *L. minor* outperformed *H. verticillata* in removing all heavy metals tested. Duck achieved the highest removal efficiency of 99.8% for nickel at 1 ppm on the 21st day and 99.7% for lead at 0.5 ppm. The highest Cd removal efficiency was 92% at 0.5 ppm on the 14th day (Table 1). These results are consistent with those of a study by [20], who confirmed the ability of duckweed to accumulate and absorb Cd. In contrast, *H. verticillata* recorded lower removal efficiencies, with the highest removal rate being 89.19% for nickel at 10 ppm on day 14, 76.67% for lead at 10 ppm on day 14, and 72.16% for cadmium at 0.5 ppm on day 21 (Table 2). These results explain the variations in plant uptake capacity, as Cd removal efficiency in *Hydrangea* declined sharply at higher concentrations due to the saturation of binding sites on the plant surface, consistent with the findings of [21]. Statistical analysis confirmed significant differences (L.S.D. = 4.26, $p \leq 0.05$) in the metal interactions with time and concentrations for duckweed, although a few cases did not show any significant differences. Conversely, for *H. verticillata*, the analysis revealed significant differences among all metal interactions (L.S.D. = 0.227, $p \leq 0.05$), except for two specific groups of interactions. Overall, the results indicate that increasing the exposure duration increases the removal efficiency [22], while higher initial concentrations of Ni and Pb improve the removal efficiency in *H. verticillata* owing to an increased driving force for translocation [23]. These results are consistent with those of several previous studies that indicated that *H. verticillata* and *L. minor* plants possess efficient heavy metal uptake mechanisms [24,25,26].

Table 1. Removal efficiency of *L. minor* for heavy metals (Cd,Pb and Ni) during experiment period.

Metal	1day	7 day	14 day	21 day
Cd 0.5 ppm	48.6	91.0	92.0	0.0
Cd 2ppm	68.9	81.2	89.3	0.0
Cd 4 ppm	73.1	83.5	89.5	0.0
Pb 0.5 ppm	16.3	85.0	91.5	99.7
Pb 5 ppm	21.5	97.5	97.7	99.0
Pb 10 ppm	8.5	94.5	97.0	97.3
Ni 1 ppm	48.3	80.1	93.2	99.8
Ni 5 ppm	42.3	52.7	62.5	63.4
Ni 10 ppm	28.7	44.7	45.3	46.5

Table 2. Removal efficiency of *H. verticillata* for heavy metals (Cd,Pb and Ni) during experiment period.

	1 day	7 day	14 day	21 day
Cd 0.5 ppm	4.7	20.7	59.0	72.2
Cd 2 ppm	3.3	7.8	28.6	35.2
Cd 4 ppm	0.7	30.0	55.9	60.7

Pb 0.5 ppm	7.3	35.3	60.7	76.7
pb 5 ppm	6.0	18.0	28.0	71.3
pb 10 ppm	28.3	41.0	76.7	76.7
Ni 1 ppm	16.0	32.4	44.6	75.0
Ni 5 ppm	4.7	21.3	21.6	80.0
Ni 10ppm	13.7	27.0	89.2	89.2

7.2. Biological Concentration Factor (BCF)

The lowest biological concentration factor (BCF) for *L. minor* cadmium metal was 596 mg/l at 2 ppm on the first day, while the highest value recorded was 10534 mg/l at 0.5 ppm on the 14th and 21st days. The statistical analysis showed that there were important differences in how the metal and time interacted, with a probability of L.S.D. = 462 and $p \leq 0.05$, as detailed in Table 4-1, Appendix 2). The highest value of BCF of Nickel was 383568 mg/l at 1 ppm on the 21st day, while the lowest value was 167 mg/l at 10 ppm on the 7th day. The statistical analysis, with a probability (L.S.D. = 17342, $p \leq 0.05$), indicates that there were important differences in all interactions between the metal and time, as shown in Table 3. The highest BCF value for *L. minor* of lead was 277278 mg/l at 0.5 ppm on the 21st day, and the lowest value was 507 mg/l at 10 ppm on the first day. The statistical analysis showed that there were significant differences in how the metal interacted with time, except at 5 and 10 ppm, where no significant differences were observed (Table 3).

It could be suggested that plants may be more effective at absorbing Cd at low concentrations because *L. minor* can accumulate the metal at low concentrations (0.5 ppm). This advantage might be due to the plant's capacity to tolerate low concentrations of metal poisons[27]. High concentrations (2 ppm) resulted in a decreased capacity of the plant to absorb Cd in comparison to lower concentrations, although there was a decrease on the first day. This may be because Cd initially slows down the plant's absorption of heavy metals, which can negatively affect the plant at the beginning of the experiment [28].

The highest BCF of Ni was (383568 mg/l) at 1 ppm on the 21st day. This result implies that plants can acquire a large amount of Ni at 1 ppm, particularly on the 21st day. This result might indicate that the plants can adapt to Ni, as evidenced by the noticeable increase in BCF over time [29]. The lowest BCF (167 mg/l) was observed on the first day at 10 ppm. After seven days, the higher concentration (10 ppm) of Ni demonstrated a lower ability to accumulate metal compared to Ni at 1 ppm. This variation could be explained by the fact that plants may have a lower capacity to absorb metals when exposed to high concentrations of Ni. Plants may not absorb Ni effectively at high concentrations, which lowers BCF values [30].

The results showed the highest BCF for Pb (277278) was observed at 0.5 ppm on the 21st day. These results were similar to those for Cd and Ni, indicating that *L. minor* has a significant capacity to absorb Pb at 0.5 ppm, especially on day 21. This result occurred because Pb may accumulate more efficiently at low concentrations than at high concentrations. The plant might have been free to create more potent defenses or absorption strategies as a result of the longest period of exposure (21 days) [31]. As a lead metal, it first generated a poor response. This might be related to the early harmful effects of high lead exposure during the initial growth stages of plants. According to this response [32], *L. minor* may initially struggle to absorb lead at high concentrations.

Table 3. Bioconcentration factor value (BCF) (mg/l) of heavy metal in *Lemna minor* during experiment period.

Day	Cd (0.5 ppm)	Cd (2 ppm)	Cd (4 ppm)	Ni (1 ppm)	Ni (5 ppm)	Ni (10 ppm)	Pb (0.5 ppm)	Pb (5 ppm)	Pb (10 ppm)
1 day	1149±365	596±39	884±66.5	810±125.8	421±14167±21	1501±66.4	900±4	507±9.7	
7 day	8773±1748	1259±102	2361±129.4	4737±537	451±20288±12	27420±5402	10345±98.6	17037±689	
14 day	10534±6132	2018±12.4	1750±50.1	8478±159	693±62415±74	59666±17998	12488±122.6	9294±252	
21 day	0±0	0±0	0±0	383568±71304	404±1	338±19277278±148346	11068±2411	11513±3115	
L.S.D. Day		533		20027			42013		
L.S.D. Con.		462		17344			36384		

Table (4) shows the Biological Concentration Factor (BCF) for *H. verticillata* for cadmium. The highest value was 738.8 at 0.5 ppm on the 21st day, whereas the lowest value recorded was 14.47 at 2 ppm on the first day. The Biological Concentration Factor (BCF) for *H. verticillata* exposed to nickel was highest at 425.44 at 1 ppm on the 21st day, while it was only 0.56 at 5 ppm on the first day (Table 4). The highest value of BCF for *H. verticillata* exposed to lead was 877.56 at 0.5 ppm on the 21st day, and the lowest value was 0.83 at 5 ppm on the first day (Table 4). The statistical analysis showed significant differences in all metal interactions during the experiment, except for a few specific cases: the 0.5 ppm treatment of Cd with the 0.5 ppm treatment of Pb, the 2 ppm treatment of Cd with the 4 ppm and 10 ppm treatments of Pb, and the 5 ppm and 10 ppm treatments of Ni with the 4 ppm treatment of Cd and the 5 ppm and 10 ppm treatments of Pb. The details are presented in Table 4.

Plants that are beneficial for phytoremediation can absorb many heavy metals through their roots, handle different kinds of metals, and change their functioning in response to environmental changes [33]. The effectiveness of a plant's phytoremediation capacity can be evaluated using the bioaccumulation factor (BCF)[34]. Metal availability, media pH, chemical composition of co-pollutants, and interactions with organisms in the rhizosphere all affect plant pollutant uptake [35]. These findings support the phytoremediation potential of these plants for most metals, which is consistent with previous research [36].

The bioconcentration factor (BCF) is a relative indicator that determines how well plants can accumulate metals compared to the concentration of these metals in the surrounding environment. BCF is an indicative parameter for estimating the suitability of plants for the phytoremediation of metals [37]. Higher BCF values (>1000) indicate better suitability of plants for the remediation of contaminants from the environment [38, 39]. Pandey [37] found that high BCF values in *Azolla caroliniana* plants improved their ability to remove Cu and Zn from the environment. These results are consistent with those of [37]. Plants with high BCF values are hyperaccumulators that can remediate even small amounts of contaminants by extracting them. This finding is consistent with that of [40], who found that *H. verticillata* had the highest BCFs for copper and zinc. The study revealed that the tested *H. verticillata* plants effectively removed Cd and Pb from the environment. This is because they have high BCF values, even when the metals are present in small amounts.

Table 4. Bioconcentration factor value (BCF) (mg/l) of metal (Cd, Ni, and Pb) in *H. verticillata* during experiment period.

	1 day	7 day	14 day	21 day
Cd 0.5 ppm	38.66	43.9	109.8	738.8
Cd 2 ppm	14.47	15.7	36.18	99.16
Cd 4 ppm	15.07	17.7	35.84	86.45
Ni 1 ppm	2.54	6.14	15.19	425.44

Ni 5 ppm	0.56	1.19	7.78	171.70
Ni 10 ppm	0.82	1.93	5.91	190.46
Pb 0.5 ppm	3.62	10.71	56.60	877.56
Pb 5 ppm	0.83	1.85	8.37	99.21
Pb10 ppm	4.08	10.18	11.11	65.77
L.S.D		41.49		

7.3. Total Chlorophyll

The total chlorophyll content in *L. minor* plants exposed to Cd decreased slowly in all treatments over the experimental period compared to that in the control group. The highest value was 3.3 mg/g in the 0.5 ppm treatment on the first day, and the lowest value was 0.61 mg/g in the 4 ppm treatment on the 14th day. (Figure 2). The *total chlorophyll levels of L. minor* increased on the seventh day when exposed to high concentrations of (Ni) compared to the control group. However, they decreased on the fourteenth and twenty-first days, with the highest level being 5 ppm (5.35 mg/g) on the seventh day and the lowest level being 10 ppm (1.01 mg/g) on the twenty-first day (Figure 3). All treatments showed an increase in the *total chlorophyll levels of L. minor plants* on the first day compared to the control. They then decreased over the next seven, fourteen, and twenty-one days, except for 5 ppm on the seventh day and 10 ppm on the fourteenth. The highest level was 10 ppm (4.99 mg/g) on the first day, and the lowest was 0.5 ppm (1.16 mg/g) on the 21st day (Figure 4). Statistical analysis was performed using a probability ($p < 0.05$) and showed significant differences. Statistical analysis was performed using the least significant difference (LSD) test at a significance=0.13, $p \leq 0.05$, showed that there were changes in concentration and time compared to the control in all metal treatments, except for 5 ppm and 10 ppm of lead, which did not show any significant changes.

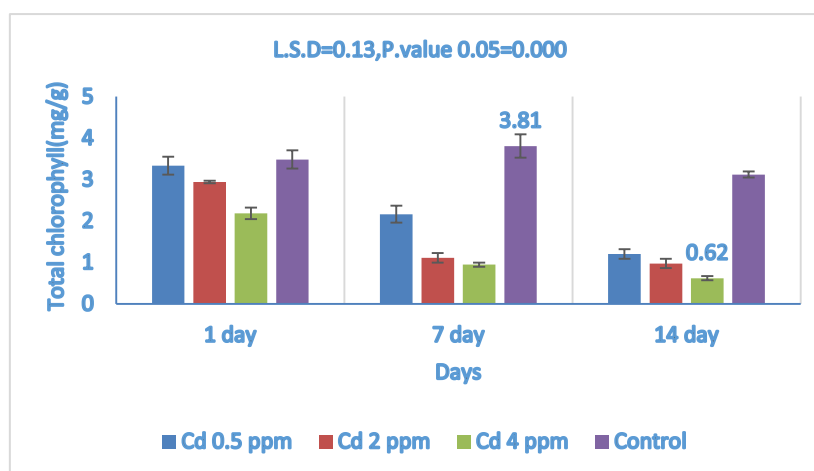


Figure 2. Effects of (Cd) on total chlorophyll of *L. minor* during the experimental period.

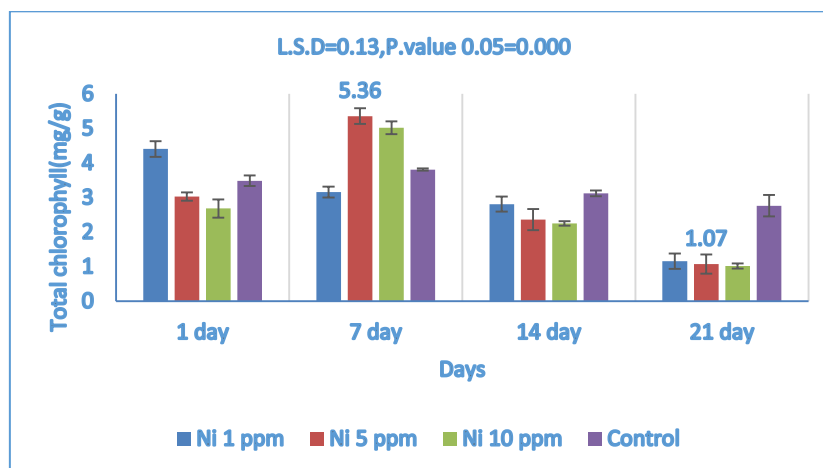


Figure 3. Effects of (Ni) on total chlorophyll of *L. minor* during the experimental period.

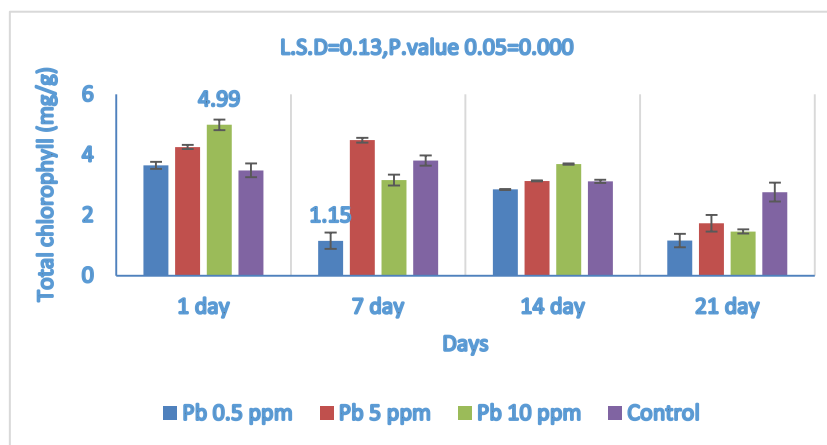


Figure 4. Effects of (Pb) on total chlorophyll of *L. minor* during the experimental period.

The total chlorophyll of *H. verticillata* is shown in Figure 5, where the total chlorophyll after exposure to Cd metal demonstrates a decreased in all treatments during the experimental period compared to the control, especially on the 14th and 21st days. The total chlorophyll content of the plants after exposure to Ni metal increased on the 7th and 14th days and decreased on the 21st day, except at 5 ppm concentration. The highest value was recorded in the 5 ppm treatment on the 7th day, which was 13.48 mg/g. The lowest value was recorded in the 10 ppm treatment, which was 0.61 mg/g on the first day (Figure 6). The total chlorophyll of the plant, after being exposed to Pb metal, showed an increase on the 7th and 14th days in all treatments except for 5 ppm at the 14th day, followed by a decrease on the 21st day. The highest value recorded was 15.64 mg/g in the 0.5 ppm treatment on the 7th day. The lowest value was recorded in the 10 ppm treatment, which was 3.9 mg/g on the 1st day (Figure 7). The statistical analysis of total chlorophyll revealed significant differences (L.S.D. = 0.68, P.value $\leq$ 0.05) between time and concentration for all study metals, except the control with 1 ppm of nickel and all treatments of cadmium with each other. and 0.5 ppm of lead with 5 ppm of Ni (Figures 5-7). Kumar et al.[41] also noted that high concentrations of Ni are toxic, leading to decreased chlorophyll content, oxidative stress, and inhibited plant growth.

The results indicated that the overall amount of chlorophyll decreased throughout the experiment, and the levels of chlorophyll a, b, and carotenoids showed the same pattern when the plants were exposed to heavy metals such as Cd, Ni, and Pb. This decrease occurred because heavy metals, especially Cd, harmed the plants by lowering chlorophyll levels, reducing photosynthesis, and affecting carbon fixation [42]. Heavy metals can alter the permeability of substances through mitochondrial membranes and reduce the function of enzymes that clear reactive oxygen species (ROS) in the plant's antioxidant system, leading to an overproduction of ROS. Such events can also occur due to damage to chlorophyll molecules[43] or suppression of manufacturing-related enzymes. Weak absorption of iron and magnesium is crucial for chlorophyll production. [44]. These hazardous metals can inhibit the critical enzymatic activity involved in vital plant functions, such as respiration, photosynthesis, and water uptake.[45]. Cd and amino levulinic acid block the enzymes that produce chlorophyll and protochlorophyll reductase. While total chlorophyll increased in the basins exposed to lead and nickel on days seven and fourteen of the experiment, it decreased on day twenty-one. This could be because the plants were not sensitive to heavy elements during those days, and some research has indicated that the decrease in chlorophyll content following heavy element treatment is caused by a decrease in carotenoids[46]

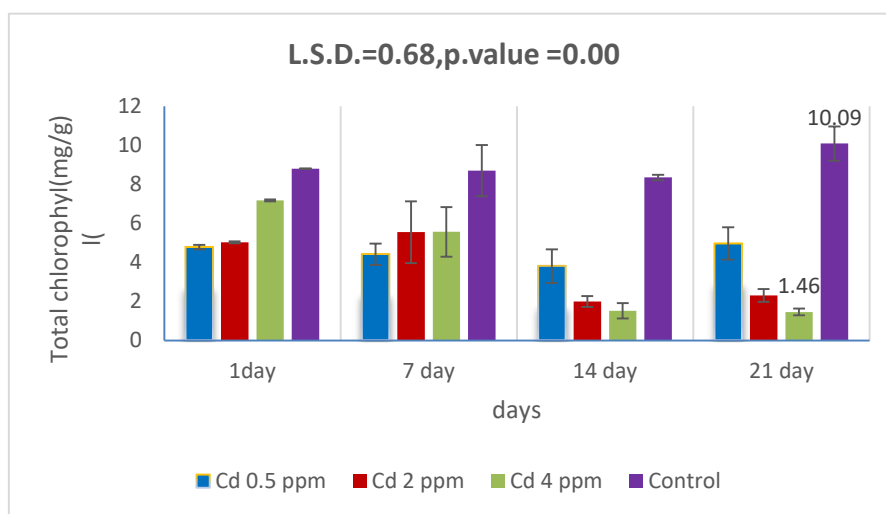


Figure 5. Effects of Cd+2 on total chlorophyll of *H.verticillata* during study experiment.

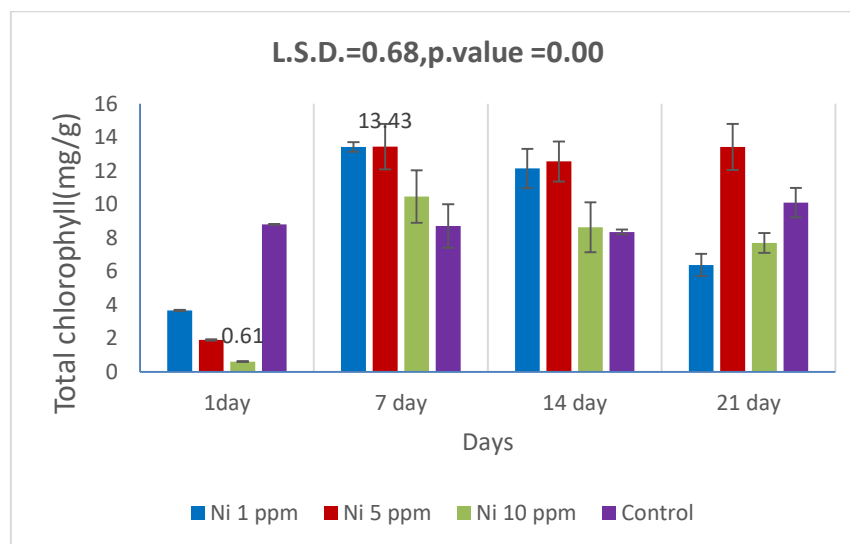


Figure 6. Effects of (Ni) on total chlorophyll of *H. verticillata* during study experiment.

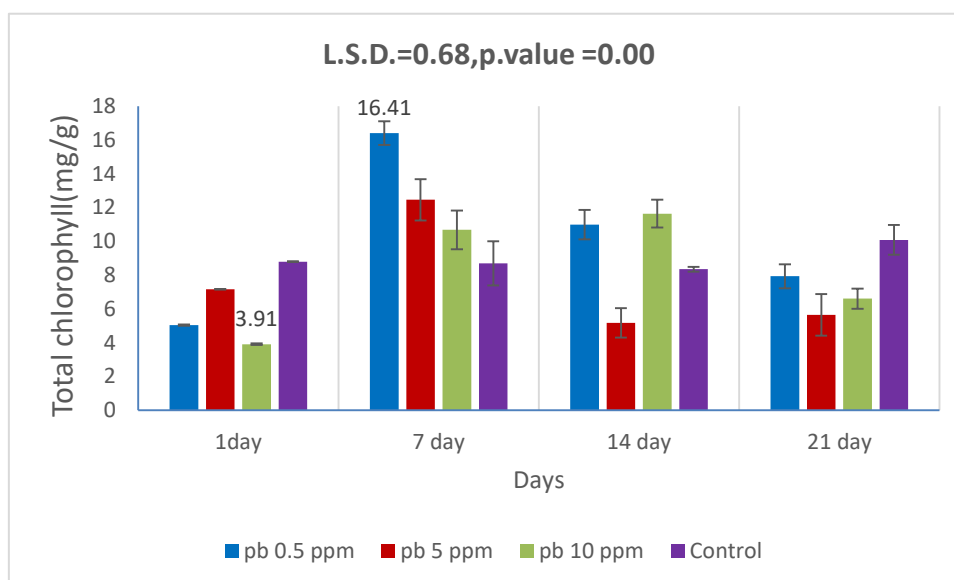


Figure 7. Effects of (Pb) on total chlorophyll of *H. verticillata* during study experiment.

7.4. Biomass

Lemna minor showed a clear response to Cd, with biomass significantly reduced at all concentrations compared to the control (29.75 g/L). The effect was more severe in *H. verticillata*. Biomass decreased significantly, especially at 4 ppm, where the value (0.017 g/L) became very close to zero, indicating a severe toxic effect and almost complete growth cessation. All nickel concentrations caused a decrease in *L. minor* biomass compared to the control, but the effect was gradual with increasing concentration, with the lowest value being 14.38 g/L at 10 ppm. *H. verticillata* biomass decreased significantly with increasing nickel concentration, with the lowest value being 2.02 g/L at 10 ppm, indicating that nickel also affects the growth of this plant. *Lemna minor* had a less pronounced effect on Pb than on the other metals. Although there was a decrease in biomass at 5 ppm, the values at 0.5 ppm and 10 ppm were relatively close to the control

value (Table 5), which may indicate that this plant exhibits greater resistance to lead. Although all lead concentrations decreased the biomass of *Hydrilla verticillata* compared to that of the control, the effect was not as severe as that of Cd. Cd and Ni were the most toxic metals, causing statistically significant reductions in biomass in both plants at all concentrations.

Lead had a lesser effect on *L. minor*, with a significant effect at only one concentration (5 ppm). In contrast, Pb had a significant effect on *H. verticillata* at all concentrations.

Based on the LSD value, *H. verticillata* showed greater sensitivity to the toxic effects of metals, with the effect being significant across all three metal treatments. *L. minor*, on the other hand, showed relative resistance to lead.

The biomass of the chosen plants for phytoremediation technology is essential to achieve an elevated removal rate of hazardous heavy metals. Certain plant species can accumulate significant levels of heavy metals in their tissues; however, they exhibit poor biomass production and slow growth rates, rendering their application in phytoremediation impractical. A study by [47] demonstrated a reduction in the biomass production of *Brassica napus* cultivated in Cd-contaminated soil. The biomass of the chosen plants for phytoremediation technology is essential to achieve an elevated removal rate of hazardous heavy metals. This study selected *H. verticillata* because of its substantial biomass, rapid growth rate, and ability to remove heavy metals from polluted water. The plants showed vitality in both the control and aquarium with low metal concentrations, whereas the plants grown in water contaminated with high levels of Pb and Cd exhibited leaf yellowing that progressed to browning. This experiment revealed significant disparities in weight and length among plants cultivated in enclosures supplemented with different metal concentrations. It appears that (Cd) was more effective in reducing plant biomass than (Ni) and (Pb). Heavy metals, such as Cd, have varying effects on the growth and metabolism of *H. verticillata*. High Cd levels caused *H. verticillata* to grow poorly, produce less biomass, and exhibit visible signs of damage similar to those observed by other researchers in different plants. The reduction in biomass was significantly greater when excess Cd was present, followed by Ni and Pb, which had a lesser impact than the control treatment. This reduction may represent the cumulative physiological function under stress conditions. The observed decrease in biomass production due to metal stress agrees with that reported earlier for *H. verticillata* [8]. When plants are exposed to high metal concentrations, they lose enzymes required for metabolism [48], photosystems [49], and the ability to use minerals [50].

Table 5. Biomass (g/L) of *Lemna minor* and *H. verticillata* treated by heavy metals (Cd, Pb, and Ni) during experiment period.

Metal type	<i>L. minor</i>	SD±	<i>H. verticillata</i>	SD±
Cd 0.5 ppm	16.83	2.5	3.24	1.7
Cd 2 ppm	12.65	2.47	0.84	1.3
Cd 4 ppm	14.02	2.64	0.017	0.9
Ni 1 ppm	16.46	2.33	7.66	2.1
Ni 5 ppm	15.91	0.80	5.04	1.3
Ni 10 ppm	14.38	0.85	2.02	0.72
Pb 0.5 ppm	26.11	0.80	9.54	4.4
Pb 5 ppm	19.18	4.23	8.52	2.67
Pb 10 ppm	25.92	5.12	10.43	2.4
Control	29.75	1.68	16.38	3.2
L.S.D	4.3		3.66	

Conclusion

This study successfully demonstrated that both *L. minor* and *H. verticillata* possess significant potential for the phytoremediation of heavy metals from aqueous solutions. A comparative

analysis revealed that *L. minor* significantly outperformed *H. verticillata* in the removal efficiency of all tested metal, with its effectiveness supported by exceptionally high Bioconcentration Factor (BCF) values. The superior metal accumulation capacity of *L. minor*, particularly for nickel and lead, positions it as a highly promising and efficient biological tool for environmental remediation.

Furthermore, the research showed that exposure to high concentrations of metals induced physiological stress in both plant species, as evidenced by a notable decline in biomass and total chlorophyll content. Cd was the most toxic of the tested metals, causing the most severe reduction in biomass.

This study provides a robust foundation for the application of *L. minor* as a viable and effective natural solution for treating heavy metal-contaminated water, especially in the context of aquatic environments in Iraq.

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Detection of Environmental Risks Resulting from Burning Medical Waste in Baghdad and its Impact on Public Health

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Abstract. The research involved the investigation of PAHs in the air resulting from the incinerations of medical waste, and the determination the proportions of these aromatics in the air due to a importance as high-risk pollutants. It also identifies concentrations of some toxic heavy metals such as lead (Pb^{2+}), Cadmium (Cd^{2+}), mercury (Hg), Nickel (Ni^{2+}), and zinc (Zn^{2+}) in samples of air because of their negative effects on human health. Four air samples were collected from the hospital incinerator Al-alwea, 2020, and the HPLC technique was used to identify PAHs. The results showed that hydrocarbon ratios ranged between 0.720-1.789 $\mu g / m^3$ and were within the permissible limits of WHO (1996). Heavy metals were ranged between (0.042-1436.6 $\mu g / m^3$). The zinc component was very high and its highest value was 1436.6 $\mu g / m^3$. The concentration of Nickel was also high and ranged from 0.087-1.078 $\mu g / m^3$. Mercury concentrations ranged between (0.042-0.0586 $\mu g / m^3$) and these concentrations, although few, are considered to be contaminated with air. The study concluded by examining the association between the ambient air concentrations of PAHs and heavy metals, and it found excellent agreement. This study has made significant progress in understanding this system in the study area and is an initial step toward the monitoring and Assessment for health purposes.

Keywords. Air pollution, Medical waste, Poly Aromatic Hydrocarbons, Heavy metals, Medical incinerators.

1. Introduction

Health institutions are one of the most important sources of pollution in polyaromatic compounds and others if there is negligence in the application of preventive measures, so the cleanliness of the environment of institutions is necessary diseases for the health and safety of the community as a whole as it falls within the communities, the subject of environmental pollution at present is The world's major industrial and developing countries. Because pollution is a direct threat to human health, the pollution of polycyclic aromatic compounds is a recent "one of the most important contaminants of carcinogens [1,2].

It is also known that medical waste contains toxic heavy metals such as lead, cadmium, mercury, and others. These elements are usually emitted in very small minutes and can penetrate the human lung. The presence of these elements has been a concern for environmental protection agencies and health practitioners because of their negative effects [3]. On human health due to their toxicity and environmental stability [4]. There are more than 100 PAHs that affect humans by

water, air inhalation or indirectly [5]. The Occupational Health and Safety Association (OSHA) has set a limit for polyaromatic compounds in the air ($0.2\text{mg} / \text{m}^3$) [6]. The United States Environmental Protection Agency (USEPA) has selected a compound of poly aromatic hydrocarbon (PAHs) Due to their spread and presence in the environment more than others [7,8]. Azliyana [9], conducted a study to determine the relationship between concentrations of aromatic compounds in the air and plants and found a positive relationship between air and plants through the absorption of plant organic pollutants from the environment. The researcher Ali [10], studied the distribution and spread of aromatic compounds (PAHs) and the heavy metals produced by the Kirkuk oil refinery products using the HPLC technology as well as the atomic absorption technique for determining concentrations of pollutants. The current research aims to measure the multi-ring aromatic compounds in air samples and determine the concentrations of heavy metals resulting from burning medical waste in Al-alwea Hospital in Baghdad and its impact on public health.

1.1. Study Area

The study area (Al-always Hospital) is located in the center of Baghdad, between 33.304047N and 44.459641E, Figure 1. In Iraq, there were 294 government hospitals, 49 private hospitals, 37 private hospitals, and 208 other health institutions. The medical services sector is in charge of providing health care to the citizens through the services it provides in diagnosis, examination, and treatment. This substantial and vital sector includes health institutions such as government and private hospitals, health centers, health laboratories, blood banks, etc. [11].

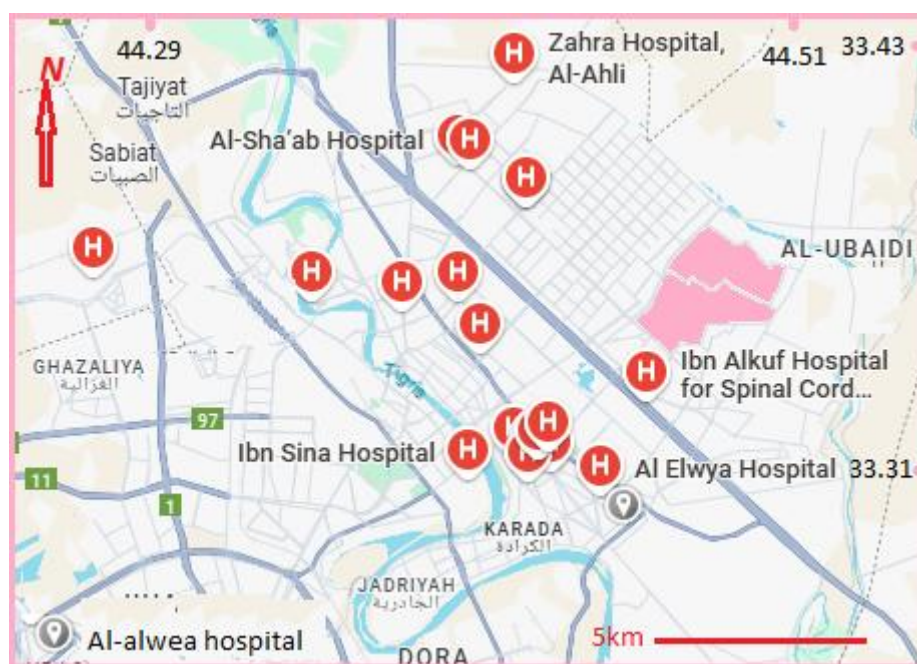


Figure 1. Location of study area with some medical services in Baghdad city.

2. Materials and Methods

In July 2020, Eight air samples were collected from four locations close to the Alawiya Hospital Incinerator in Baghdad, which is believed to be contaminated with polyaromatic compounds and Heavy metals. All the sample sites are shown in Table 1.

Table 1. The sites and the symbols of samples collected in the study area.

Sites	Symbol	Details
Site 1	S1	About 8m away from the waste incinerator/Right
Site 2	S2	About 16m away from the waste incinerator/Right
Site 3	S3	About 8m away from the waste incinerator/Left
Site 4	S4	About 16m away from the waste incinerator/Left

2.1. Preparation of Standard Solutions for Polycyclic Aromatic Hydrocarbons

1. The aromatic compounds were set using a German HPLC device of SYKUM-type origin.
2. Prepare a standard solution for each polycyclic aromatic compound with different concentrations (0.5,1,2,4,8,10ppm).
3. 20µl of the standard solution is injected into the C18 column of the HPLC.
4. Use an improvised phase (40% acetonitrile - 60% water) and flow rate (1.2ml / min) at the temperature of the column equal to (30C) and the wavelength of the detector was fixed at 254nm.
5. The time of arrest for each aromatic compound was determined and the peak of each aromatic compound was determined.
6. The concentrations of these standard polyaromatic compounds were determined against the peak height values of each compound obtained from the device.

2.2. Preparation of Air Samples

Air models were collected by the Air Volume Sampler (Sniffer) air collection device to collect airborne minutes. Before starting to collect samples, the Cellulose Filters are dried at 80 ° C to remove the moisture. Then weigh it before placing it in the filter holder and record the weight, which represents (W1). When the sample collection process is started in the area to be studied, the filter is placed in the device (in the filter holder). The device is operated and calculated at start-up and the stability of the ball within the airflow size scale. The size of the air is indicated by the iron ball, which represents (V1) and the device is usually for half an hour, and after the time calculated the size of the new air, which is marked by the ball of iron, which represents (V2). Then the candidate is taken and weighed again, where the new weight (W2) is calculated.

$$\text{Suspended particle } SP = \frac{(W2-W1)}{Vt} \times 10^6$$

whereas:

SP: Concentration of suspended nuclei measured in µg / m³ .

W1: The weight of the filter before the withdrawal is measured in grams (g)

W2: The weight of the leak after the withdrawal is measured in grams (g)

Vt: Volume of drawn air in cubic meters (m3)

106: converting the gram into the microgram

The size of the drawn air Vt is calculated according to the following equation:

$$Vt = \frac{Qt}{1000}$$

whereas:

Q: Airflow rate in liters per minute

Total modeling time per minute

Q is calculated from the following equation

$$Q = \frac{V1+V2}{2}$$

whereas:

V1: The volume of air indicated by the iron ball at the start of the machine

V2: The air volume indicated by the iron ball at the end of the machine.

2.3. Method of Extracting Air Samples

The multi-ring aromatic compounds are extracted from the fiber filter by a separating funnel where it is placed with 10ml of methanol and dichloromethane mixture and 1: 1 after that, the shaker is applied to the shaker for 24hr Multivariate aromatic compounds in the solvent mix. After extraction, the models are concentrated.

Method of digesting air models to measure heavy elements:

1. Fold the air filter in contrast to the contents of the suspended minutes and then cut the filter into small pieces and put it in the Teflon Baker
2. Place 5ml of HNO₃ acid at 65% concentration in the baker, warming the baker gradually so that it stays approximately 1ml.
3. Add 3 ml of HClO₄ acid at 75% concentration to the baker container on the model and heat until the 1 ml remains in the form.
4. Add 3ml of HF acid with heating until 1ml remains. The sample is filtered in a 50ml volume vial and the model is ready for measurement.

2.4. Contamination Factor

The contamination factor (CF) is a simplistic and useful tool for observing the contamination of heavy metals [13]. The CF is a geochemical indicator applied to define the contamination level from the heavy metals. The contamination factor (CF) is the ratio of metal concentration in the air sample and in the Earth's crust (background), respectively. The values of background were possessed by Kabata-pendis [13], as Follows: Table.

Table 2. Reference dose RFD (mg/kg-day) for each heavy metal within the dermal contact pathway.

Heavy metals	(Pb ²⁺)	(Cd ²⁺),	(Hg)	(Ni ²⁺),	(Zn ²⁺)
Reference dose RFD (mg/kg-day)	0.15	15	0.08	8.40E-01	70

CF = Concentration of metals in dust sample /Concentration of metals in background.

CF values are categorized into 4 classes as follows (14): (i) Low contamination, CF < 1 (ii) Moderate contamination, 1 < CF < 3 (iii) Considerable contamination, 3 < CF < 6.

3. Results and Discussion

After the separation process, eight polycyclic aromatic compounds were obtained for four Sites: Naphthalene, benzo (k) Fluoranthene, Fluoranthene, Benzo (a) Anthracene, Phenanthrene, Acenaphthene and Benzo (b) Fluoranthene. These compounds were concentrated after attending a standard solution of aromatic compounds (0,05,1,2,4,8,10ppm) and analyzed by HPLC, where the concentrations of these standard multichannel compounds were measured against peak height values for each compound obtained from the device as in Figuer.2.

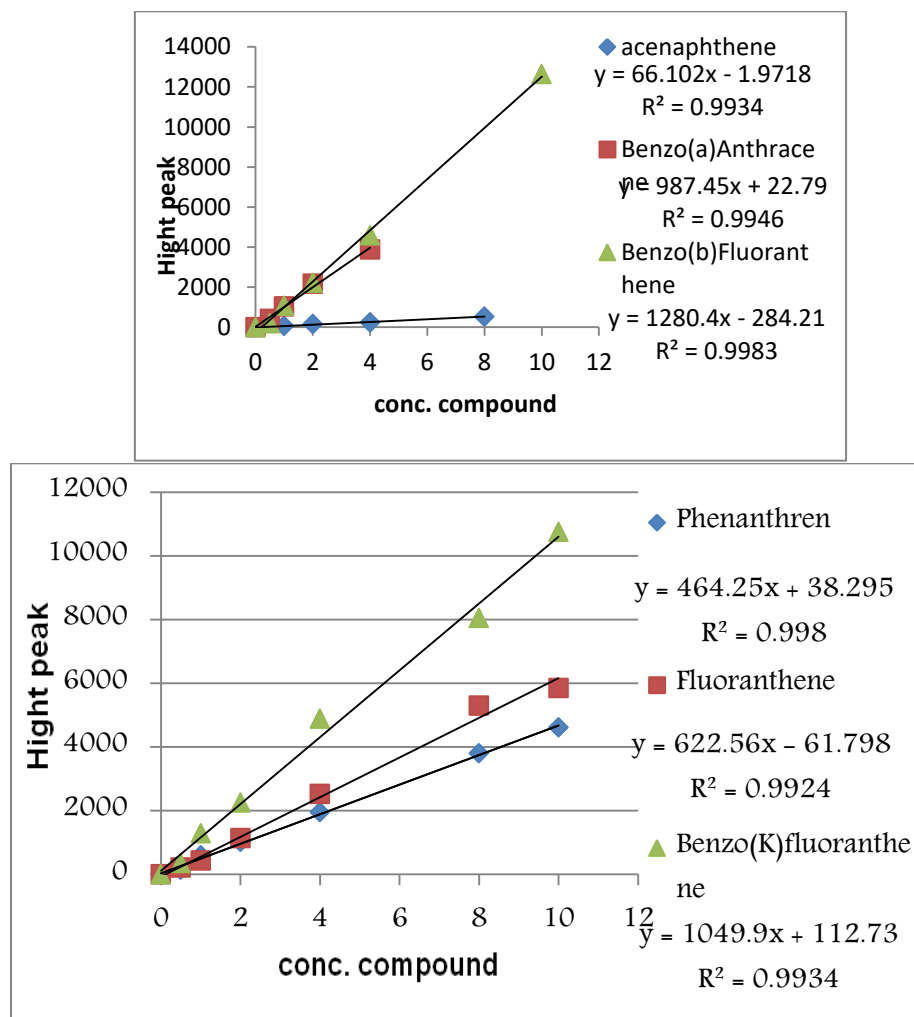


Figure 2. The linear relationship between high peaks and concentrations of the following polycyclic aromatic hydrocarbons: acenaphthene (z), naphthalene (o), benzo (a) anthracene, benzo (b) fluoranthene, benzo (k) Fluoranthene, Phenanthrene.

Table .3 shows that the concentrations of aromatic compounds from the separation process of the Sites are not high and that the total concentrations of aromatic compounds for each filter range from 0.720-1.789 $\mu\text{g} / \text{m}^3$. The total (ΣPAH) in the study area (minimum and maximum) is less than the permissible limit when compared with the permissible limit of 0.2 mg / m^3 [15]. These compounds are among the main causes of cancer and one in 100,000 people is diagnosed with cancer, even when the amount of these compounds is less than the permissible limit [16].

Table 3. Concentrations of Polyaromatic compounds in the air produced by the medical incinerator

PAHs	Ambient air Concentration ($\mu\text{g}/\text{m}^3$)			
	S1	S 2	S 3	S 4
Naphthalene	0.618	1.0393	-	-
Benzo (k) Fluoranthene	0.102	-	-	-
Phenanthrene	-	0.1599	-	-
Benzo(a)Anthracene	-	-	1.119	-

Acenaphthene	-	-	0.339	-
Fluoranthene	-	-	-	0.55
Benzo(b)Fluoranthene	-	-	-	1.239
Total	0.720	1.1992	1.458	1.789

In this study, Heavy metals were measured using the atomic absorption device after digesting the models of the air filters withdrawn from the medical incinerator of the hospital. Table 4, shows that there was contamination of the lead element and ranged between (0.989-1.9615 $\mu\text{g}/\text{m}^3$) When compared with the 0.5 $\mu\text{g}/\text{m}^3$ limit [15].

Table 4. Concentrations of heavy metals in the air produced by the medical.

Heavy metals	Ambient air Concentration ($\mu\text{g}/\text{m}^3$)			
	S1	S2	S3	S4
Hg	0.042	0.0443	0.0482	0.0586
Ni	0.087	0.0872	0.0896	1.078
Pb	0.989	1.26	1.78	1.9615
Zn	860.1	1038.6	1211.27	1436.6
Cd	BDL	BDL	BDL	BDL

The table above also, shows that there is also contamination of the nickel (Ni) element with a concentration range is 0.087-1.078 $\mu\text{g}/\text{m}^3$. This concentration is high when compared to the 0.2 $\mu\text{g}/\text{m}^3$ according to the World Health Organization [15], and mercury ranges between 0.042-0.0586 $\mu\text{g}/\text{m}^3$. There are no specific sources that determine the acceptable percentage of pollution in the air according to the recognized organizations, but there is a source [17]. The value of mercury in the air is about 30.02 $\mu\text{g}/\text{m}^3$. Zinc has a very high concentration, 1436.6 $\mu\text{g}/\text{m}^3$ in the S4 camper with S1 Finally, all the measured values of Cadmium (Cd) below the detection limit which 0.01, Figure 3.

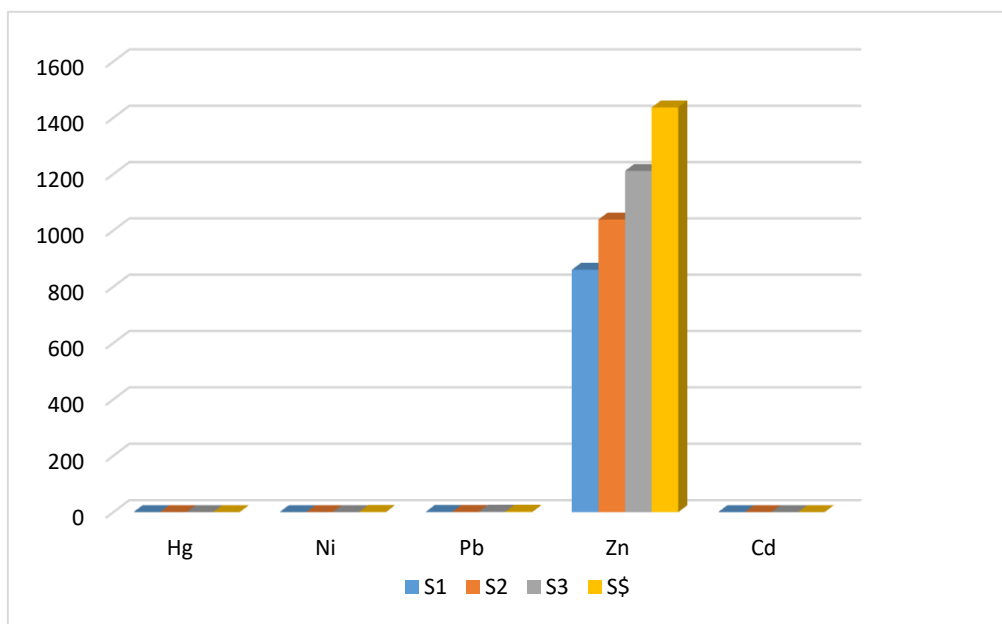


Figure 3. The concentration of heavy metals in all samples of the study area.

The heavy metal contamination in the air (all sites) was assessed with the determination of the CF (Table 5). The CF values illustrate the contamination varies between low and very high. The mean Pb and Zn values represent very high contamination while Ni and Hg are the low

contamination. the very high contamination values are registered in the Pb and Zn. This is due to human activities and may also be waste emissions.

Table 5. The contamination factor (CF) calculated in the air for heavy metals in the study area.

Elements	CF S4	CF S3	CF S2	CF S1
Pb	8.4	6.593	13.07	11.86
Cd	0	0	0	0
Hg	0.525	0.6025	0.55375	0.85
Ni	0.00103	0.0011	0.0128	0.0010
Zn	12.28	14.83	20.522	17.30

Lastly, this study examined the association between the ambient air concentrations of heavy metals and PAHs. Figure 4. The results discovered a high degree of agreement. Since the overall pattern of changes in heavy metal concentrations was found to be rather similar to that of PAHs, it was possible to conclude that the two pollutants came from the same source.

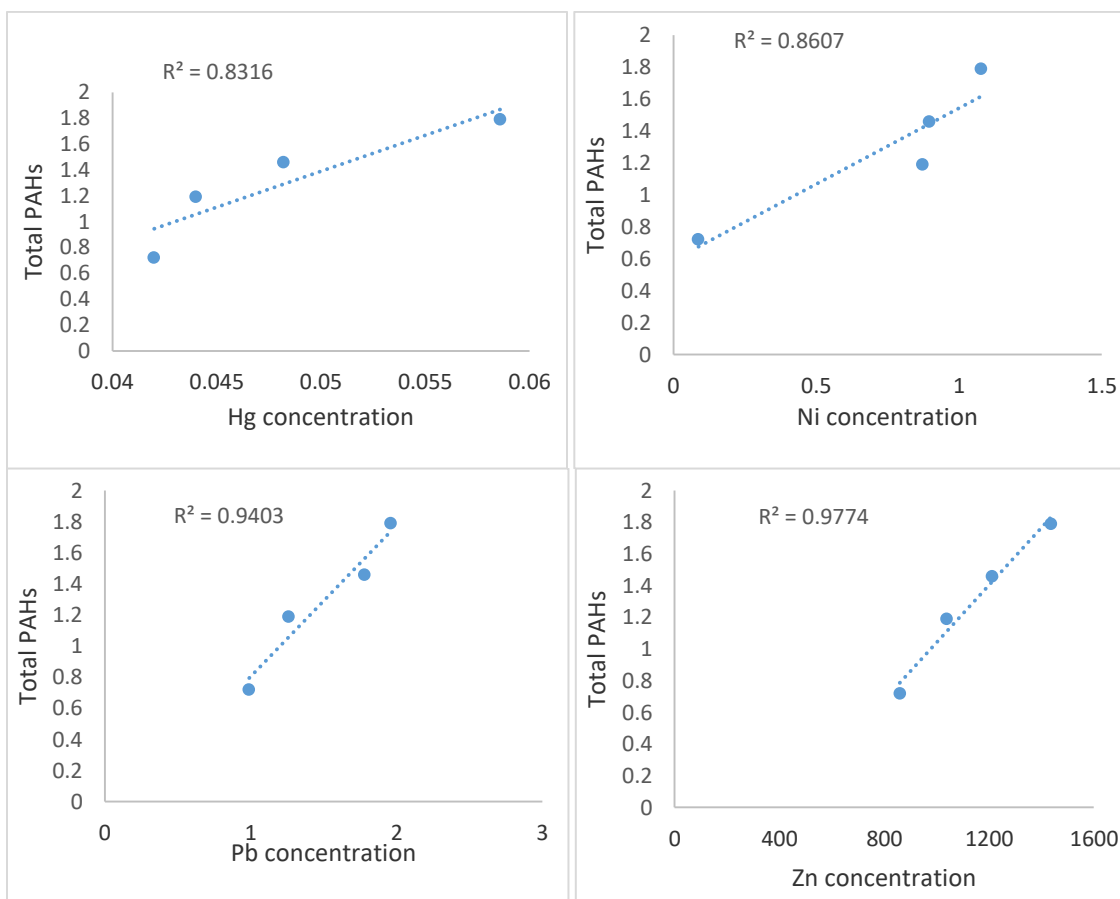


Figure 4. The relation between PAHs and Heavy metals (Hg, Ni, Pb and Zn) in the study area.

Conclusions

1. The results of the research show that the air in Baghdad is contaminated with polychromatic compounds resulting from the burning of medical waste.
2. Medical Waste Air pollution with heavy elements and high rates.
3. Hospitals and health centers have a significant role in environmental pollution in Baghdad.
4. Lack of awareness of the seriousness of this pollution by workers in the field of medical waste management.
5. The subject of the research needs to be studied more and more time and to pay great attention to the importance of this issue by officials in hospitals where we could not get many models to give comprehensive results with all details of environmental pollution.
6. The lack of cooperation of the management of several hospitals in taking samples of medical waste offered except Alawi Hospital.

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Detection and Identification of Proteins Produced by Blue-Green Algae Using GC-MASS

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Abstract. The current study included the isolation, identification, and purification of local algae, especially *Phormidium mucicola*, *Oscillatoria pranceps*, and *Anabaena variabilis*, which belong to the phylum Cyanobacteria. These algae were collected from the Tigris River in Baghdad City and were isolated, cultured, and propagated on BG-11 culture medium for the purpose of growth, propagation, and protein extraction. Protein concentrations in the algal isolates reached 5.030, 4.940, and 3.190 mg L⁻¹, respectively, with biomass of 0.2347, 0.0963, and 0.3732 mg L⁻¹. Optimal environmental conditions for growth and propagation were provided, including light intensity of 300 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ and a temperature of 25 $\pm$ 2°C, to ensure efficient algal cell division and biomass production for protein yield. The results of the current study showed that the selected algae possessed a notable biochemical content in terms of both biomass and protein concentration for each isolate. The results of the GC-MASS (Gas Chromatography–Mass Spectrometry) assay showed multiple protein isoforms within the extracted algal biomass. For *Oscillatoria pranceps*, identified compounds included C₁₃H₂₈, C₂₄H₃₈O₄, and C₂₀H₄₂ with molecular weights of 184, 390, and 284, respectively. *Anabaena variabilis* exhibited compounds such as C₁₆H₃₂O₂ and C₁₆H₃₂O, with molecular weights of 256 and 240, respectively. *Phormidium mucicola* showed the presence of C₁₈H₃₄O₂, C₂₀H₄₀O₂, C₁₀H₂₀O, C₁₂H₂₂O₂, and C₁₆H₂₂O₄, with molecular weights of 282, 312, 156, 198, and 278, respectively. These results indicate the promising potential of these local cyanobacterial strains as a source of biochemically valuable substances, especially proteins, for use in biotechnological applications.

Keywords. Blue-green algae, Proteins, Biomass, Cultured, and Propagated.

1. Introduction

It is estimated that the global population will reach 10 billion within three decades, resulting in an estimated 70% increase in food demand. This projection aligns with data from the Food and Agriculture Organization (FAO), which anticipates a doubling of global meat production by 2050, highlighting the urgent need for novel food sources and alternative dietary strategies[1]. Furthermore, 30% of the planet's terrestrial resources are associated with livestock farming, which lead to numerous environmental problems. Animal-derived proteins, particularly meat and

dairy products, are among the most consumed and nutritionally complete. However, their increased demand cannot be sustained due to their low conversion rate and substantial environmental impact [2].

Consequently, the search for alternative protein sources is not only essential from a nutritional and safety standpoint but must also be evaluated through the lens of environmental sustainability [3]. As a result, several alternative protein sources have been explored, including insect, microbial, plant, and algal proteins [4]. Among them, Algal biomass is a potential non-animal protein source, considered a suitable alternative due to its numerous advantages such as improved growth rates, minimal freshwater use, non-competition with arable land, carbon-neutral emissions, bio-remediation potential, and the potential to synthesize various bioactive compounds [5].

The term "algae" encompasses a wide set of autotrophic, aquatic, non-embryonic organisms, including both prokaryotic (blue-green algae) and eukaryotic species with simple reproductive structures. They can be unicellular, colonial, filamentous, or comprised of simple tissues [6]. Microalgae are ubiquitous single-celled organisms and highly adaptable, thriving in diverse ecosystems [7]. Despite their advantages, commercial-scale microalgae production for food applications remains limited, due to most of their production relies on batch cultivation systems, which involve low yield, high harvesting and processing costs, and variability in product quality-factors that hinder their market integration. For human consumption, the following species, *Arthrospira*, *Chlorella*, *Aphanizomenon*, *Dunaliella*, and *Haematococcus*, are the most widely used, although not all are confirmed by the European Food Safety Authority (EFSA), which restrict they're in Europe [8]. Therefore, the current study aimed to detect proteins produced by some blue-green algae using GC-Mass.

2. Materials and Methods

2.1. Algal Identification

The identification of algal species was performed using a hemocytometer slide under 400× magnification with a compound light microscope. Several taxonomic references for algal identification were used [9-12].

2.2. Cultivation and Propagation of Algae Used for Treatment

Three algal isolates from the Iraqi aquatic environment were used: *Phormidium mucicola*, *Oscillatoria pranceps*, and *Anabaena variabilis*. The isolates were grown in a modified BG-11 culture medium, with a 1-liter culture per isolate, under suitable environmental conditions of $25 \pm 2^\circ\text{C}$ and a light intensity of $300 \mu\text{mol m}^{-2} \text{s}^{-1}$, at a light: dark ratio of 16:8.

2.3. Algal Isolation

Isolation was performed using the following steps:

- Prepare BG-11 medium supplemented with 2% agar. After autoclaving the medium, it was poured into sterile Petri dishes and left to solidify for use in isolation. A few drops of the water sample to be cultured were spread on the surface of the solid medium. Plates were incubated under regulated illumination conditions of $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a temperature of $25 \pm 2^\circ\text{C}$ for 7–14 days. Emerging algal colonies were microscopically examined and subculture onto fresh BG-11 agar plates under the same conditions to establish unialgal cultures. This process was repeated until pure cultures were obtained [13].

- Once a single algal species was confirmed, a portion of the colony was aseptically transferred to 250 ml Erlenmeyer flasks containing sterile liquid BG-11 media. The flasks were incubated under the same environmental circumstance for 2 weeks to promote optimal algal growth. Cultures were microscopically monitored for contamination and renewed biweekly to maintain culture viability.

2.4. Purification of Algal Isolates

Two methods were employed to obtain a pure axenic culture (free from bacteria and fungi contamination):

First: The [14] method for purifying algae involves the following:

- 50 ml of the algal culture was taken and left in the dark for 24 hours at room temperature. Then, 10 ml was withdrawn into a fresh sterile culture medium and left in the dark for 2-3 hours.
- The culture was centrifuged (2000 rpm) for 3 minutes.
- The supernatant was discarded, and the sediment was washed again with sterile distilled water. This process was repeated 10-15 times to ensure the removal of contaminants.
- A small portion of the sediment was planted in a sterile culture medium to stimulate growth.

Second: A drop of the previous culture was streaked onto nutrient agar medium and incubated at 37°C for 18 hours to confirm absence of bacterial growth.

2.5. Protein Extraction

Protein extraction was conducted following the described by [15], with slight modifications. 250 mg of dried algal biomass (for each species) was dissolved in 10 mL of 95% ethanol. The mixture was shaken for 60 minutes in an orbital shaker at 25°C and 70 rpm. After incubation, the solution was centrifuged at 6000 rpm for 15 minutes to remove cellular debris. The supernatant was placed in a clean tube and centrifuged again at 6000 rpm for 10 minutes. This extraction process was repeated three times using the same methods. The combined ethanol extracts were subjected to solvent evaporation using a rotary evaporator at 40°C to obtain the crude protein extract in the form of a dark green powder. To further purify the protein, 2 mL of distilled water was added to the residue and mixed thoroughly. Then, 5 mL of methylene chloride was added, and the mixture was shaken well to form two separate layers. The organic layer was separated using a separating funnel and concentrated at room temperature to obtain the protein content.

2.6. Protein assay

[16] method was used to determine the protein concentration of blue-green algae (Table 1).

Table 1. Calculating of bovine serum albumin (BSA) concentration using a stock solution of BSA (100 μ g ml⁻¹).

Tube No.	Volume of BSA solution (ml)	Volume of D.W. (ml)	Final volume (ml)	Final concentration (μ g/ml)
1	0	1.0	1	0
2	0.1	0.9	1	10
3	0.2	0.8	1	20
4	0.3	0.7	1	30
5	0.4	0.6	1	40
6	0.5	0.5	1	50
7	0.6	0.4	1	60
8	0.7	0.3	1	70
9	0.8	0.2	1	80

Tube No.	Volume of BSA solution (ml)	Volume of D.W. (ml)	Final volume (ml)	Final concentration ($\mu\text{g/ml}$)
10	0.9	0.1	1	90
11	1.0	0	1	100

2.7. Protein Estimation Solutions

Solutions were prepared according to [16] as follows:

1. Sodium bicarbonate solution (Na_2CO_3) 2% (Prepared by dissolving 10 g of Na_2CO_3 in 500 ml of 0.1 M NaOH)
2. Sodium tartrate solution 2% (Prepared by dissolving 2 g of sodium tartrate solution in a volume of distilled water, then making up the volume to 100 ml).
3. Copper sulfate solution ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) 1% (Prepared by dissolving 1 g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 100 ml of distilled water).
4. A mixture of Solutions (2) and (3) in a 1:1 (v/v) ratio, this mixed prepared immediately before use.
5. A mixture of Solutions (4) and (1) in a 50:1 (v/v) ratio, this mixed prepared immediately before use.
6. Folin–Ciocalteu solution: A commercially available reagent, diluted with distilled water at a 1:2 (v/v) ratio.
7. Bovine serum albumin (BSA) solution was prepared at a concentration of 0.125 g ml^{-1} and used to generate the standard calibration curve for determining total protein content (Fig.1).

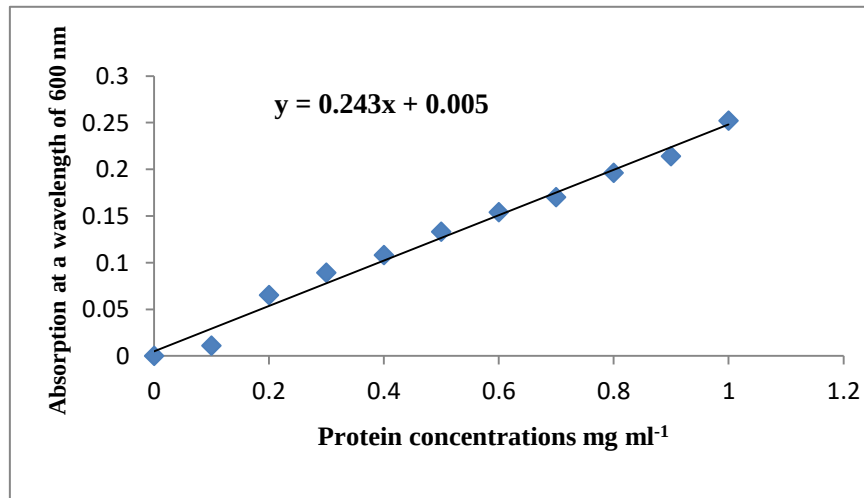


Figure 1. Protein measurement using BSA and a standard calibration curve.

2.8. Detection of Conjugated Compounds in *Spirulina algae* Extract Using GC-MASS

Hydrocarbon compounds were examined using a GC-MS (Shimadzu 2010 plus), was performed on a DB-1 column, thickness 0.25 mm, maximum temperature 340°C , 30M, and a thermal program ($85 = 2 \text{ min}$), 10°C/min , ramped to 300°C , a total flow inside the column of 10 ml/m , and a pressure of 100 kPa, with injection volume of sample at $5 \mu\text{L}$ [17]. The chromatogram and detected compounds are shown in Figure 2.



Figure 2. GC-MS chromatogram.

3. Results

Proteins were extracted from the biomass of each algal isolate following the propagation and harvesting processes. The results indicated that *Phormidium mucicola* yielded a protein concentration of 5.030 mg L⁻¹ from a biomass of 0.2347 mg L⁻¹ in a 1-liter BG-11 liquid culture. Similarly, *Oscillatoria pranceps* produced 4.940 mg L⁻¹ of protein from a biomass of 0.0963 mg L⁻¹, while *Anabaena variabilis* yielded 3.190 mg L⁻¹ of protein from a biomass of 0.3732 mg L⁻¹ under identical conditions (Table 2, Fig. 3).

Table 2. Protein content estimates derived from microalgae.

Algal isolation	Biomass (mg L ⁻¹)	Protein (mg L ⁻¹)	Volume (L)
<i>Phormidium mucicola</i>	0.2347	5.030	1
<i>Oscillatoria pranceps</i>	0.0963	4.940	1
<i>Anabaena variabilis</i>	0.3732	3.190	1

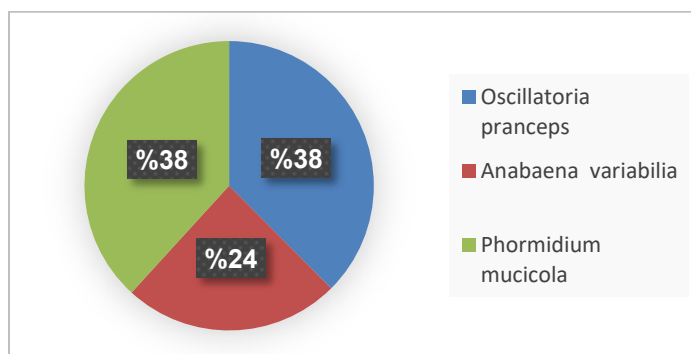


Figure 3. Protein content ratios in selected local algae isolates.

The culture was monitored by examining the optical density using an atomic spectrometer at a wavelength of 545 nm from the first day of culture (acclimatization phase), until the ninth day of culture (end of the exponential growth phase), to determine the extent of algal cells' absorption of the surrounding medium. [18] also indicated that temperature variations can affect the timing of algal growth phases. Temperature variations significantly influenced the onset of the exponential growth phase; he observed temperature, at 22°C, the exponential phase commenced on fifth day,

whereas at 26°C, it began on seventh day. The algal biomass also increases directly with elevated nitrogen-nitrate concentrations in the culture medium. Notably, an increase in nitrate concentration relative to phosphate led to a more substantial biomass accumulation in some cyanobacterial species, attributed to their rapid uptake of both phosphate and nitrogen. The nitrogen content in algal cells typically ranges between 6.5- 8.3% of their dry weight under standard conditions, though it may decrease under nitrogen-deficient scenarios and increase when nitrogen is abundant. This underscores the critical role of nitrogen in algal growth, often surpassing the requirement for phosphate (Fig. 4).



Figure 4. Shows the algal culture selected at this study during the development and propagation period.

The results of GC-MASS analysis revealed multiple protein formulas within the extracted biomass of the selected algal isolates. For *Oscillatoria pranceps*, the detected compounds included C_3H_{28} , $C_{24}H_{38}O_4$, and $C_{20}H_{42}$, with respective molecular weights of 184, 390, and 284 Da. In *Anabaena variabilis*, the primary identified compounds were $C_{16}H_{32}O_2$ and $C_6H_{32}O$, with molecular weights of 256 and 240 Da, respectively. While, *Phormidium mucicola* exhibited a broader range of bioactive compounds, including $C_{16}H_{22}O_4$, $C_{16}H_{22}O_4$ (isomeric structure), $C_{12}H_{22}O_2$, $C_{10}H_{20}O$, $C_{20}H_{40}O_2$, and $C_{18}H_{34}O_2$, with molecular weights of 282, 312, 156, 198, and 278 Da, respectively (Fig. 5, 6, and 7 and Tables 3, 4 and 5).

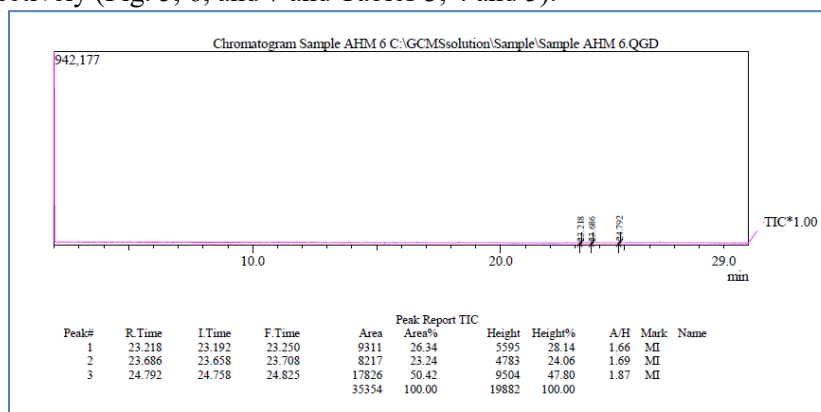
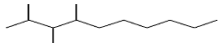
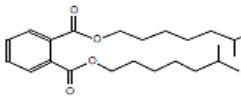


Figure 5. GC-MASS analysis of *Oscillatoria pranceps* algal proteins.

Table 3. Protein composition of *Oscillatoria pranceps* algae by GC-MASS analysis.

Concentration %	Molecular weight	Structural composition of the compound	Structural formula	Compound name	Retention time (min)	Algae
26.34	184		C ₁₃ H ₂₈	2,3,4-Trimethyldecane	23.218	<i>Oscillatoria pranceps</i>
23.24	390		C ₂₄ H ₃₈ O ₄	Diisooctyl phthalate	23.686	
50.42	282		C ₂₀ H ₄₂	Eicosane	24.792	

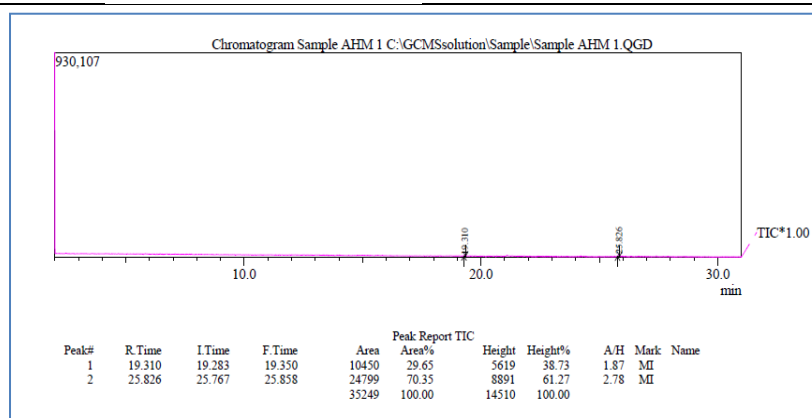
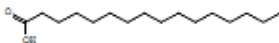


Figure 6. Protein profile of the alga *Anabaena variabilis* analyzed using GC-MS Spectrometry.

Table 4. Protein content of *Anabaena variabilis* as identified by GC-MS analysis.

Concentration %	Molecular weight	Structural composition of the compound	Structural formula	Compound name	Retention time (min)	Algae
29.65	256		C ₁₆ H ₃₂ O ₂	Pentadecanecarboxylic acid	19.310	<i>Anabaena variabilis</i>
70.35	240		C ₁₆ H ₃₂ O	Palmitaldehyde	25.826	

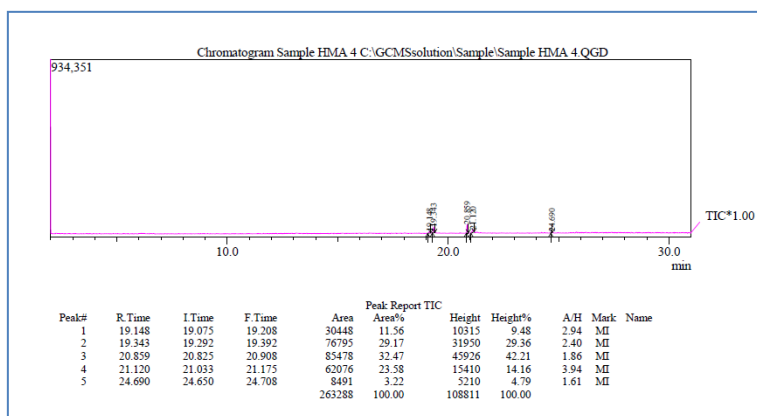
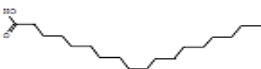
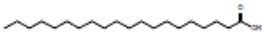
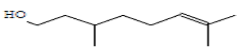
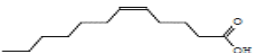
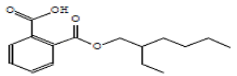


Figure 7. Protein profile of the alga *Phormidium mucicola* analyzed using GC-MS Spectrometry.

Table 5. Protein content of *Phormidium mucicola* as identified by GC-MS analysis.

Concentration %	Molecular weight	Structural composition of the compound	Structural formula	Compound name	Retention time (min)	Algae
11.56	282		$C_{18}H_{34}O_2$	delta.(Sup9)-cis-Oleic acid	19.148	<i>Phormidium mucicola</i>
29.17	312		$C_{20}H_{40}O_2$	Arachidic acid (synthetic)	19.343	
32.47	156		$C_{10}H_{20}O$	Dihydrogeraniol	20.859	
23.58	198		$C_{12}H_{22}O_2$	cis-5-Dodecenoic acid	21.120	
3.22	278		$C_{16}H_{22}O_4$	Phthalic acid, mono-(2-ethylhexyl) ester	24.690	

*Ester compound Unsaturated $C_{20}H_{40}O_2$

4. Discussion

The protein composition of microalgae varies significantly based on factors such as digestibility and essential amino acid content. While many plant-based and some animal-derived proteins lack certain essential amino acids, such as histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine [19]. Microalgae often provide a more complete amino acid profile, enhancing their nutritional value. Furthermore, the protein produced from some microalgae is found in higher concentrations than animal and plant proteins, and their peptides are biologically active and have been used as antioxidants to lower blood pressure and prevent atherosclerosis [20]. Figure 8 illustrates the types used in the current study. Notably, cyanobacteria like *Anabaena variabilis* and *Phormidium mucicola* have demonstrated high protein content and favorable amino acid compositions, making them promising sources of bioactive peptides with potential health benefits.

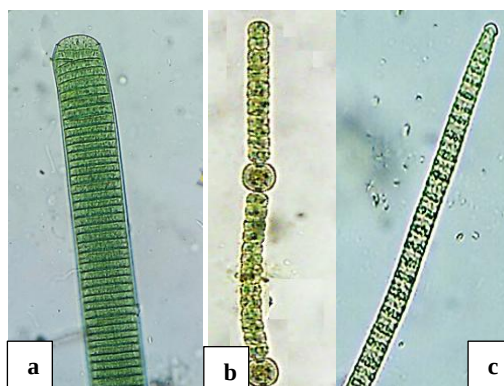


Figure 8. Algae species used in the study: (a) *Oscillatoria pranceps*, (b) *Anabaena variabilis*, (c) *Phormidium mucicola*.

Table 2 shows that the alga *Oscillatoria pranceps* produces three types of compounds, with Eicosane ($C_{20}H_{42}$) being the most abundant, constituting approximately 50.42% of the total identified compounds. Eicosane, a hydrocarbon commonly found in various plants plays an important role in bio-metabolism. Some studies also indicate that Eicosane has an effective antimicrobial activity against pathogenic microbes [21].

Table 4 shows that *Anabaena variabilis* exhibited a high concentration of Palmitaldehyde ($C_{16}H_{32}O$) as its concentration reached 70.35%, and largest area of the total area of the compounds identified by the GC-mass device (Fig.6). Palmitaldehyde is considered an important enzyme, as it is an intermediate in glycosphingolipid metabolism, serving as a substrate for the synthesis of sphingosine-1-phosphate (S1P), a signaling molecule involved in various cellular processes. The presence of such metabolites underscores the metabolic versatility of *Anabaena variabilis*, which is also known for its nitrogen-fixing capabilities and potential in bioremediation applications. [22] confirmed that the genus *Anabaena* is one of the genera rich in protein materials, which amount to 43-56% of the dry weight of this genus, and it has a high capacity to fix nitrogen and remove carbon dioxide from polluted water [23]. This result was consistent with [24], observed that this genus contains several enzymes, including amylase, protease, and phosphatase.

In *Phormidium mucicola*, GC-MS analysis identified five proteinaceous compounds, with Dihydrogeraniol being the most prevalent at 32.47%, followed by Arachidic acid ($C_{20}H_{40}O_2$) at 29.17%. Dihydrogeraniol, also known as citronellol, is a monoterpene with applications in fragrance and potential antimicrobial activity. Arachidic acid, a saturated omega-6 fatty acid, plays a role in cell membrane structure and energy storage and is crucial for brain activity [25].

The current results are consistent with the study of [26], [27] that blue-green algae are very rich in secondary metabolites including bioactive peptides and biologically active pesticides and lipids with antioxidant, antimicrobial, and anticancer properties.

Conclusion

This study demonstrates that these cyanobacteria are a high-protein source, despite the variation in protein content between species, which has practical and health significance. This highlights the potential of these cyanobacteria as a sustainable source of high-value biomolecules for future biotechnological and pharmaceutical applications. Further molecular and functional analyses are required to explore the full range of their biological potential.

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