Plants as a Bio indicator for Assessing Micro plastic Contamination in Bottled Drinking Water

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Abstract: *Aquatic plant, specifically Lemna minor (duckweed), Nasturtium officinale (watercress), and Eichhornia crassipes (water hyacinth), were selected as bio indicators owing to their heightened sensitivity to pollutants and their capacity to exhibit distinct morphological and physiological alterations upon exposure to contaminants. The consumption of bottled water has witnessed a significant rise in Iraq and globally, attributed to its perceived purity; however, the plastic packaging utilized may disintegrate into micro plastics that subsequently contaminate potable water and pose threats to human health as well as ecological systems. The objective of this investigation was to assess micro plastic contamination in bottled drinking water by employing an integrated approach of spectroscopic analysis (FTIR) alongside responses from plant bio indicators. A total of ten local bottled water brands were subjected to sampling, filtration, and evaluation through fluorescence microscopy to ascertain particle morphologies, coupled with FTIR spectroscopy to identify polymer types, while aquatic plants were subjected to contaminated samples under meticulously controlled laboratory conditions and observed for growth rate, leaf pigmentation, root deposition, chlorophyll concentration, and enzymatic activity. The findings indicated that pellet-shaped particles were predominant across the majority of brands (e.g., Sama 96.1%, Al-Kawthar 91.6%), whereas fibrous particles were most prevalent in Louloua (50%) and Al-Rawiyah (71.1%). FTIR analysis corroborated the detection of polymers including ABS, PP, PET, PVC,*

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PMMA, and Cellophane; nevertheless, certain brands exhibited no identifiable micro plastics. The plant assays revealed biological responses indicative of stress: Lemna minor demonstrated a decrease in frond size and chlorophyll content, Nasturtium officinale displayed leaf chlorosis and inhibited growth in fiber-dominant samples, and Eichhornia crassipes evidenced root deposition of particles, particularly PET and ABS, alongside heightened antioxidant enzyme activity suggesting oxidative stress. Collectively, the amalgamation of plant bioindicators with spectroscopic analysis yielded compelling evidence of microplastic contamination in bottled water, emphasizing potential risks to both human health and ecological integrity while endorsing the utilization of aquatic plants as efficacious biological markers for the assessment of bottled water quality.

Keywords: Aquatic plant, Micro plastic Contamination, Bottled Drinking Water

Introduction

Aquatic plant, including *Lemna minor* (duckweed), *Nasturtium officinale* (watercress), and *Eichhornia crassipes* (water hyacinth), are extensively acknowledged as sensitive bioindicators due to their propensity to accumulate pollutants and manifest morphological and physiological alterations upon exposure to environmental contaminants. Their ecological significance renders them indispensable instruments for the detection of microplastic pollution within aquatic ecosystems, thereby complementing traditional chemical and spectroscopic detection methodologies. The consumption of bottled drinking water has emerged as a critical avenue for hydration globally, including in Iraq, attributed to its perceived purity and convenience. However, the integrity of bottled water is susceptible to contamination throughout various stages of production, storage, and distribution, potentially leading to alterations in its physical and chemical attributes (Hu et al., 2018; Turner, 2018; Veerasingam *et al*., 2021). Plastics, characterized as synthetic or semi-synthetic polymers, exhibit remarkable durability and resistance to degradation; nevertheless, they progressively fragment into diminutive particles through photochemical and oxidative mechanisms, resulting in the formation of microplastics and nanoplastics that may be ingested by various organisms (Brennecke, 2015; Sharma & Chatterjee, 2017). Documented occurrences of microplastic pollution trace back to the 1970s within marine ecosystems, yet contemporary research has corroborated their presence in freshwater lakes, rivers, wetlands, and even sources of drinking water (Eriksen *et al*., 2019; Mhiret Gela & Aragaw, 2022). Numerous investigations have underscored the ecological and health hazards associated with micro plastics, predominantly emphasizing chemical detection techniques such as fluorescence microscopy and Fourier-transform infrared (FTIR) spectroscopy (Pivokonsky, 2018; Tong, 2020). Although these methodologies are proficient in discerning polymer types and particle morphology, they fail to capture the biological ramifications of contamination. Conversely, aquatic plants furnish a biological lens through which pollutants can be observed, as they exhibit discernible changes including diminished growth, leaf discoloration, chlorophyll reduction, and root particle deposition in response to pollutants (Singh, 2021; Wilcox, 2020). The amalgamation of plant bioindicators with spectroscopic analysis consequently yields a more holistic framework for assessing microplastic contamination. In spite of the escalating global apprehension, a paucity of studies has amalgamated plant-based bio indication with spectroscopic analysis to evaluate micro plastic contamination in bottled drinking water, particularly within the context of Iraq. To address this research void, the present inquiry sought to assess micro plastic pollution in regionally sourced bottled water brands by fusing laboratory detection techniques with the responses of plant bio indicators. This dual-faceted approach affords innovative perspectives on environmental monitoring and public health, accentuating the threats posed by micro plastics while reinforcing the utility of aquatic plants as efficacious biological markers for evaluating the quality of bottled water.

Materials and Methods

-Sample Collection:

Bottled water samples were collected from ten local companies in Iraq: Aquafina, Louloua, Al-Kawthar, Sama, Al-Waha, Barada, Life, Veniza, Khor, and Al-Rawiyah. Each sample consisted of one liter of bottled water purchased directly from local markets. The bottles were stored under controlled laboratory conditions until analysis.

-Filtration and Visual Examination:

Water samples were filtered using cellulose filter papers to isolate potential micro plastic particles. The retained particles were examined under a fluorescence microscope after staining with Nile Red dye, which selectively binds to hydrophobic polymer surfaces. This method allowed for the identification of micro plastic shapes, including pellets, fibers, and fragments.

-FTIR Spectroscopy:

Fourier-transform infrared spectroscopy (FTIR) was employed to determine the chemical composition of the isolated micro plastic particles. Spectral analysis was conducted to identify polymer types such as acrylonitrile butadiene styrene (ABS), polypropylene (PP), polyethylene terephthalate (PET), cellophane, polyvinyl chloride (PVC), and polymethyl methacrylate (PMMA).

-Plant Bio indicator Assay

To integrate a biological perspective, aquatic plants were introduced as bio indicators of micro plastic contamination. Three species were selected based on their ecological relevance and sensitivity to pollutants: *Lemna minor* (duckweed), Nasturtium officinale (watercress), and *Eichhornia crassipes* (water hyacinth). Plants were cultivated in controlled laboratory conditions and exposed to water samples containing micro plastic particles Morphological and physiological parameters were monitored, including growth rate, leaf coloration, and root surface deposition. Observations were recorded over a two-week exposure period. These biological responses were compared with spectroscopic findings to evaluate the consistency between plant-based bio indication and laboratory detection methods.

-Data Analysis

Quantitative data from microscopic counts and FTIR spectra were statistically analyzed to determine the prevalence and types of micro plastics across different bottled water brands. Plant responses were qualitatively assessed and categorized according to severity of morphological changes. The integration of chemical and biological data provided a comprehensive evaluation of micro plastic contamination in bottled drinking water.

Results and Discussion

1. Micro plastic Detection in Bottled Water:

Microscopic examination of bottled water samples revealed the presence of microplastic particles in different shapes, including pellets, fibers, and fragments. Pellet-shaped particles were the most frequent in most brands, while fibers were dominant in Louloua (50%) and Al-Rawiyah (71.1%).

Table 1 presents the distribution of micro plastic shapes across bottled water brands, showing the relative percentages of pellets, fibers, and fragments.

percentage %	Total particles/1L	Fragment	Pellet	Fiber	Sample	No. Sample
54.5% pellet	22	2	12	8	**Aquafina**	**1**
50% fiber	8	1	3	4	**Louloua**	**2**
91.6% pellet	24	1	22	1	**Al-Kawthar**	**3**
96.1% pellet	52	1	50	1	**Sama**	**4**
66.6% pellet	3	1	2	-	**Al-Waha**	**5**

92% pellet	25	1	23	1	**Barada**	**6**
90.9% pellet	33	1	30	2	**life**	**7**
81% pellet	37	2	30	5	**Veniza**	**8**
89.7% pellet	39	2	35	2	**Khor**	**9**
71.1% fiber	45	1	12	32	**Al-Rawiyah**	**10**

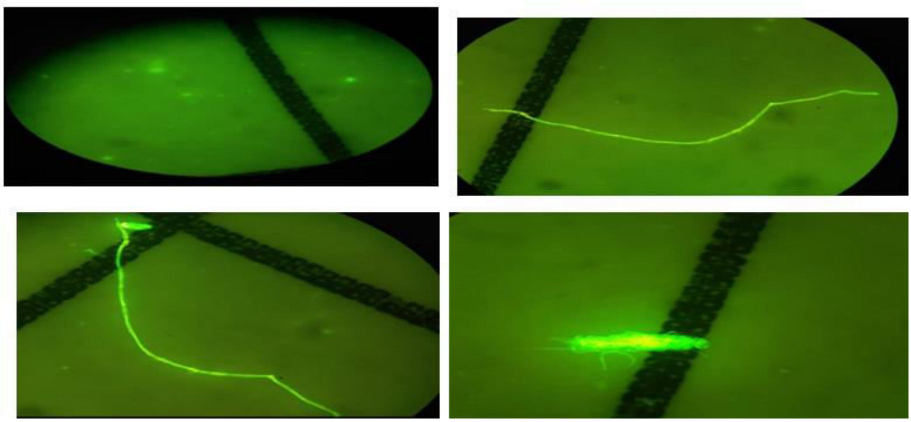


Figure (1): Micro plastic shapes under a fluorescence microscope

FTIR Spectroscopic Analysis

FTIR analysis confirmed the chemical composition of the isolated micro plastics. The polymers detected included acrylonitrile butadiene styrene (ABS), polypropylene (PP), polyethylene terephthalate (PET), cellophane, polyvinyl chloride (PVC), and polymethyl methacrylate (PMMA).

Table (2). Types of Micro plastics in Local Bottled Water

Sample name	Polymer	Absorption band(cm-1)	References
Aquafina	Acrylonitrile butadiene styrene(ABS) Nylon (all pdyamid) Cellulose acetate(CA) Polypropylene(PP) Poly (methyl methacrylate) (PMMA or Acrylic)	2922 2858 1743 840 750 624	Mhiert Gela and Aragaw,2022 Veerasingam *et al*.,2021 HU *et al*.,2018

	Polyethylene terephthalate(PET)		
Barada	Acrylonitrile butadiene styrene(ABS) Low density polyethylene (LDPE) Polypropylene(PP)	2922	Mhiert Gela and Aragaw,2022 Veerasingam *et al*.,2021
Louloua	No microplastc detected	1377	Veerasingam et al.,2021
Khor	No microplastc detected	840	
Al-Waha	Polypropylene(PP)	840	Veerasingam *et al*.,2021
Al-Kawthar	Acrylonitrile butadiene styrene(ABS) Polypropylene(PP) Polyethylene terephthalate(PET) Cellophane	2922 1375 624 840	Mhiert Gela and Aragaw,2022 Veerasingam *et al*.,2021 HU *et al*.,2018
Sama	Polypropylene(PP)	1375	Veerasingam *et al*.,2021
Life	Acrylonitrile butadiene styrene(ABS) Polypropylene(PP) Cellophane	2922 1375 840	Mhiert Gela and Aragaw,2022 Veerasingam *et al*.,2021 HU *et al*.,2018

Veniza	Polyvinyl chloride(PVC) Polypropylene(PP) Cellophane	1427 1375 840	Mhiert Gela and Aragaw,2022 Veerasingam *et al*.,2021 HU *et al*.,2018
Al-Rawiyah	Polypropylene(PP) methacrylate)(PMMA or Acrylic)	1375 750	Mhiert Gela and Aragaw,2022 HU *et al*.,2018

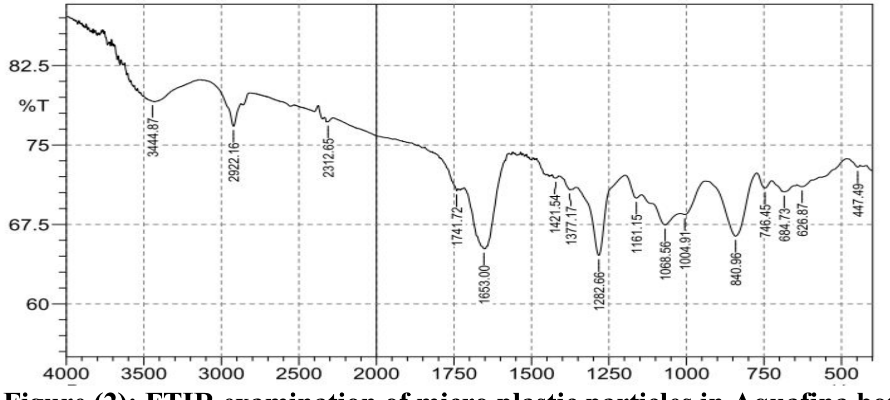


Figure (2): FTIR examination of micro plastic particles in Aquafina bottled water.

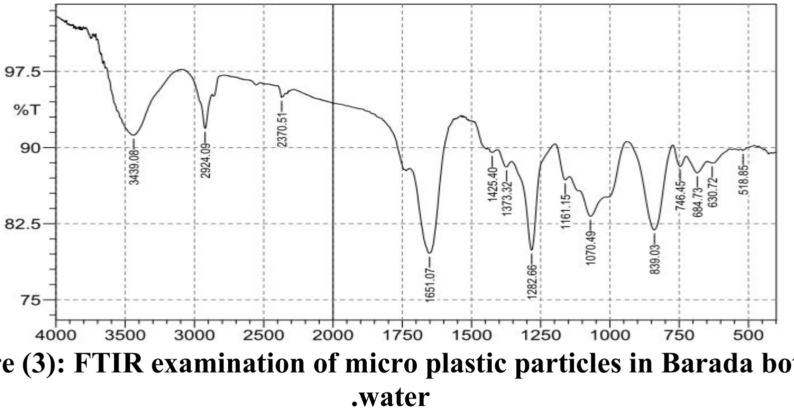
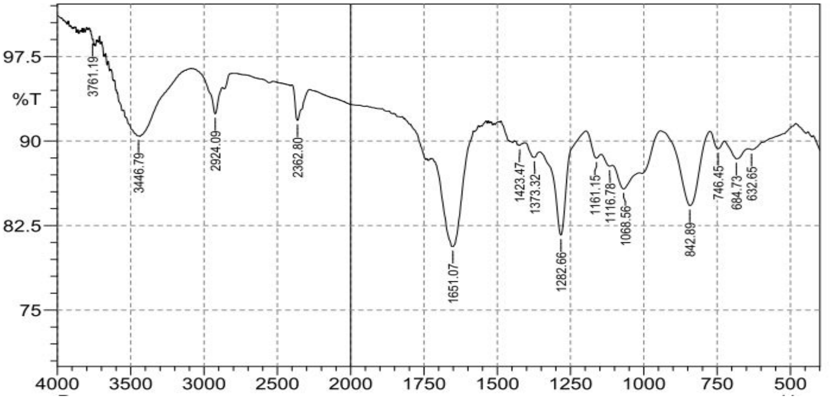


Figure (3): FTIR examination of micro plastic particles in Barada bottled .water

Figure (5): FTIR examination of micro plastic particles in Life bottled water.



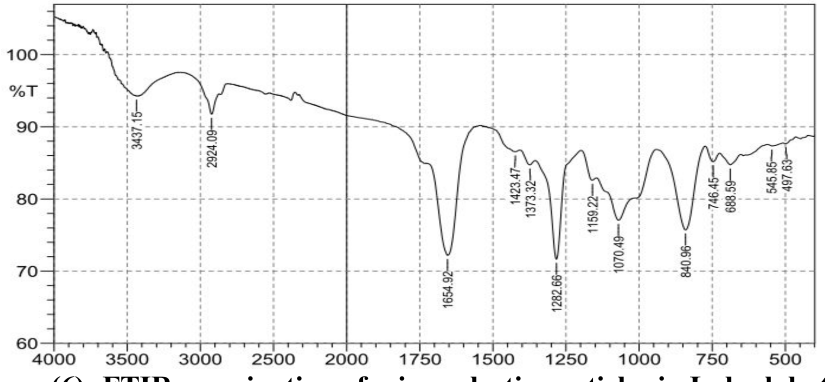


Figure (6): FTIR examination of micro plastic particles in Luluah bottled .water

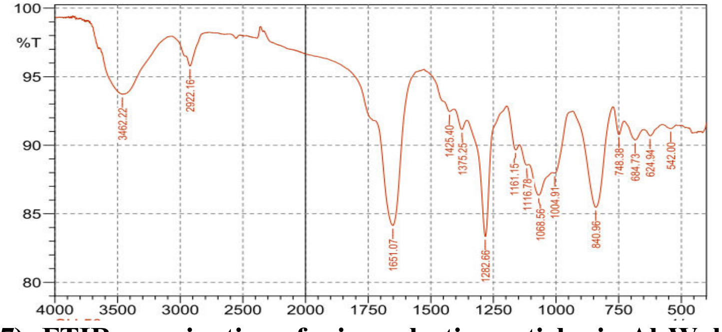


Figure (7): FTIR examination of micro plastic particles in Al-Waha bottled .water

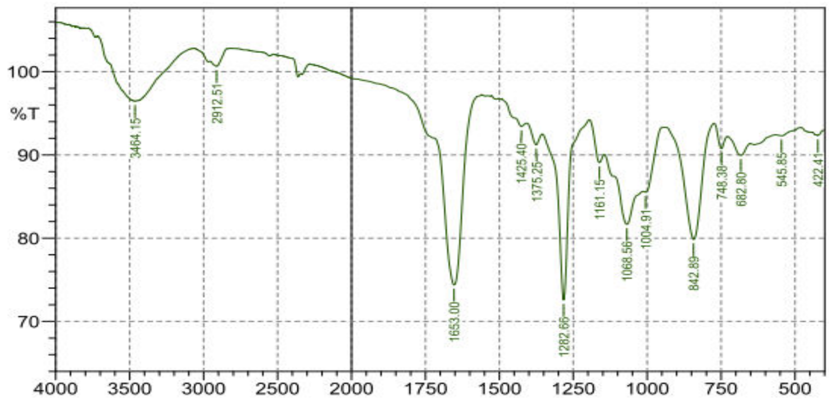
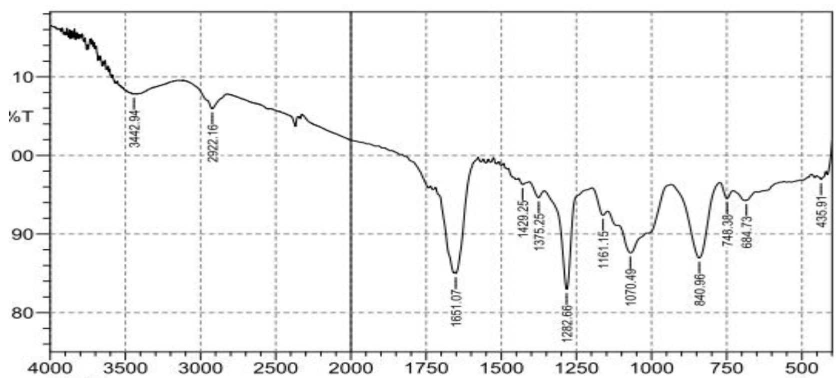


Figure (8) FTIR examination of micro plastic particles in Al-Kawthar bottled water.

Figure (9): FTIR examination of micro plastic particles in Sama bottled water.



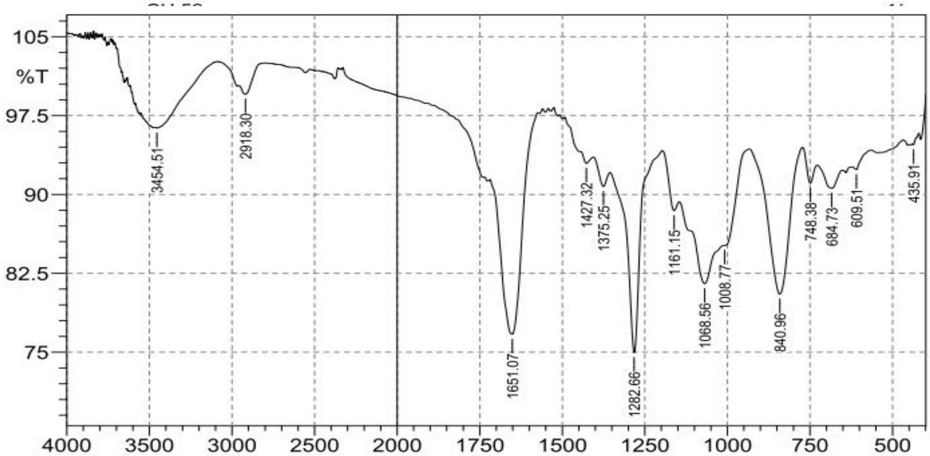


Figure (10): FTIR examination of micro plastic particle in Vineza bottled .water (second sample)

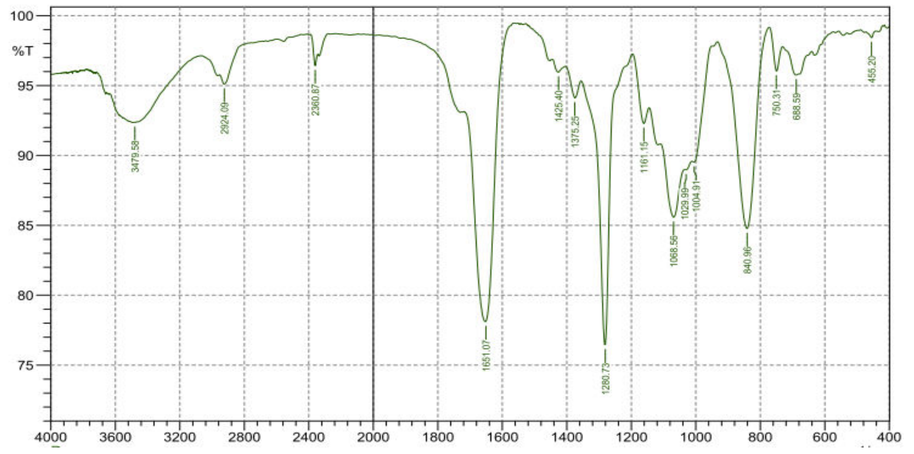


Figure (11): FTIR examination of micro plastic particles in Al-Rawiyah bottled water.

Plant Bio indicator Responses:

Aquatic plant (*Lemna minor, Nasturtium officinale, Eichhornia crassipes*) were subjected to bottled water samples containing micro plastics, and their responses were assessed through morphological, physiological, and biochemical evaluations.

- Morphological observation (Digital imaging): Comparative photographic analysis revealed a discernible decrease in frond size in Lemna minor, leaf chlorosis and wilting in *Nasturtium officinale,* and modifications in root morphology in *Eichhornia crassipes*.

- Chlorophyll content (Spectrophotometer): Chlorophyll levels were quantified at wavelengths of 645 and 663 nm. A notable decline was documented in the chlorophyll concentration of Lemna minor fronds exposed to polypropylene and acrylonitrile butadiene styrene particles (Figure 12).

- Growth rate (Biomass measurement): Plant biomass and frond count were meticulously recorded over a period of 14 days utilizing a precision balance. *Nasturtium officinale* exhibited diminished growth in samples characterized by elevated fiber concentrations.

- Microscopic root analysis (Light microscope): Microscopic scrutiny corroborated the accumulation of micro plastic particles on the roots of *Eichhornia crassipes*, particularly in samples containing polyethylene terephthalate and acrylonitrile butadiene styrene (Figure 12).

- Biochemical assays (Enzyme activity): The enzymatic activity of antioxidant enzymes (catalase, peroxidase, superoxide dismutase) was assessed using spectrophotometric methods. Increased enzyme activity was noted in plants subjected to mixed polymer contamination, suggesting the occurrence of oxidative stress

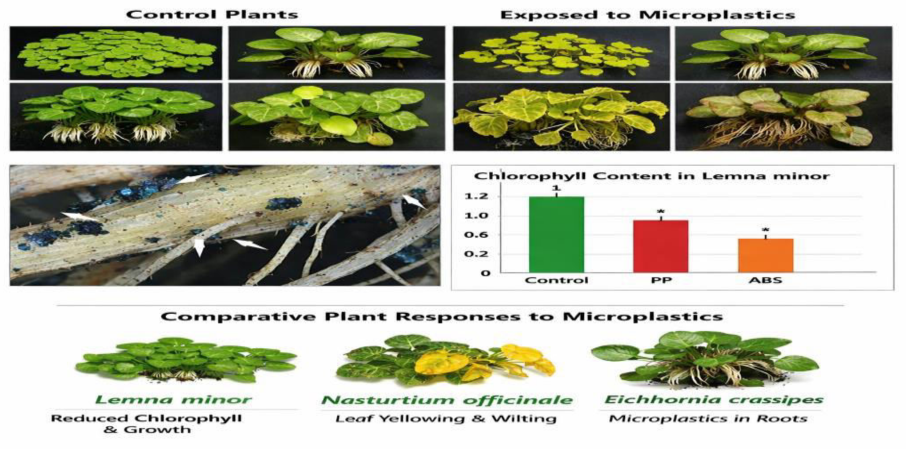


Figure 12. Plant Bio indicator Responses to Micro plastic Contamination

Discussion

The current investigation elucidates that micro plastic contamination is significantly pervasive within bottled drinking water available in Iraq, exhibiting distinct variability in particle morphology and polymer composition across different brands. Microscopic examinations indicated that pellet-shaped particles were predominantly observed in the majority of samples, surpassing 90% in brands such as Al-Kawthar, Sama, Barada, and Life, whereas fibers were found to be more prevalent in Louloua (50%) and Al-Rawiyah (71.1%). These observations align with the findings reported by Schymanski *et al*. (2018) and Wang *et al*. (2020), who similarly identified pellets as the most commonly encountered micro plastic form in bottled and treated water. Concurrently, the fiber predominance documented in specific brands parallels the observations made by Hu et al. (2018) in smaller water bodies located in China, yet diverges from the majority of bottled water studies conducted in Europe and North America, where pellets and fragments are generally more prevalent. This phenomenon suggests that regional production and packaging methodologies may exert an influence on the specific types of contamination identified. The Fourier-transform infrared (FTIR) spectroscopic analysis substantiated the presence of various polymers, including polypropylene (PP), acrylonitrile butadiene styrene (ABS), polyethylene terephthalate (PET), PVC, PMMA, and cellophane. The predominance of PP and PET corroborates global findings reported by Veerasingam *et al*. (2021), who underscored these polymers as the most prevalent in bottled water on a global scale. Conversely, the identification of ABS and PMMA is less frequently documented in studies pertaining to drinking water, suggesting potential regional disparities in packaging materials employed. Notably, the lack of detectable micro plastics in Louloua and Khor stands in contrast to the findings of Eriksen *et al*. (2019), who reported contamination in nearly all bottled water brands assessed internationally. This variation may reflect the implementation of more stringent quality control measures or differences in the selection of raw materials among local enterprises. The bio indicator assay utilizing aquatic flora yielded supplementary biological evidence of contamination. Lemna minor demonstrated a reduction in frond size and chlorophyll concentration, *Nasturtium officinale* exhibited leaf chlorosis and growth retardation in fiber-abundant samples, and *Eichhornia crassipes* manifested altered root morphology with observable particle deposition. These responses are congruent with the findings of Singh et al. (2021), who documented impaired growth and photosynthetic activity in flora exposed to micro plastics, and with Sharma & Chatterjee (2017), who delineated oxidative stress responses in organisms subjected to polymer particles. The heightened activity of antioxidant enzymes (CAT, POD, SOD) observed in this study affirms the induction of oxidative stress, while the significant root deposition in *Eichhornia crassipes* exposed to PET and ABS represents a novel contribution, as it has been less extensively recorded in extant literature. Collectively, the amalgamation of spectroscopic detection and plant bio indication enhances the robustness of the findings. The results are predominantly congruent with international studies, while concurrently illuminating distinctive local variations in particle morphology and polymer composition. This dual methodological approach not only corroborates the presence of micro plastics in bottled water but also elucidates their potential biological ramifications, thereby addressing a significant research gap within Iraq. The findings underscore the dual threat posed by micro plastics to both human health and aquatic ecosystems.

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Conclusion:

This investigation presents unequivocal evidence indicating the presence of micro plastic contamination in bottled drinking water available in Iraq, with plastic pellets emerging as the predominant particle type across the majority of brands, whereas fibrous particles were found to be more prevalent in the Louloua and Al-Rawiyah brands. The identification of multiple polymer types, notably polypropylene (PP), polyethylene terephthalate (PET), acrylonitrile butadiene styrene (ABS), polyvinyl chloride (PVC), and polymethyl methacrylate (PMMA), substantiates the assertion that packaging materials constitute a significant source of contamination. The lack of micro plastics in certain brands underscores the variability in production quality and indicates that implementing stricter manufacturing standards may mitigate contamination levels. The combination of spectroscopic analysis with bioassays utilizing aquatic plant indicators proved to be exceptionally effective, as the tested aquatic plants demonstrated morphological, physiological, and biochemical alterations consistent with exposure to micro plastics. These results not only corroborate laboratory detection methods but also highlight the ecological significance of micro plastic pollution. The documented oxidative stress and growth inhibition observed in the plants accentuate the potential threats posed to both ecosystems and human health. By integrating chemical and biological methodologies, this study addresses a significant gap in the existing micro plastic research within Iraq and contributes valuable insights to the international body of literature. The findings emphasize the imperative need for systematic monitoring of bottled water quality, enhancements in packaging practices, and further exploration of the long-term health ramifications associated with micro plastic ingestion. Subsequent investigations should aim to increase sample sizes, account for seasonal variations, and examine advanced removal technologies to alleviate contamination risks.

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