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Assessing the Biocompatibility of Ti_3C_2 MXene Nanosheets with Terrestrial and Aquatic Plant Systems: An In-Depth Analysis

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ABSTRACT

In this study, the biocompatibility of MXene, an emergent class of two-dimensional nanomaterials, with plant systems was explored. Varying concentrations of Ti_3C_2-based MXene nanosheets were administered to two distinct plant groups—soil-grown Firecracker Flowers (Crossandra infundibuliformis) and water-based Water Hyacinth (Eichhornia crassipes). Predominantly, MXene was observed to be perceived by plant systems as a contaminant, as evidenced by analysis of collected data and images during our experiement. Stronger biocompatibility with soil plants compared to aquatic plants was demonstrated. Over a 19-day observation period, no disruption to the potted soil ecosystem of Firecracker Flowers was detected due to Ti_3C_2, while a significant impact on the Water Hyacinth ecosystem was noted. The effect of Ti_3C_2 on Water Hyacinth was found to be substantial and exhibited characteristics akin to those of typical pollutants. Through advanced techniques such as optical microscopy and Scanning Electron Microscopy (SEM), the assimilation and spatial distribution of Ti_3C_2 within plant matrices were investigated. Ti_3C_2 was found to be absorbable by plants, but its diffusion within the plant body was determined to be notably limited. This study sheds light on the potential environmental implications of MXene nanomaterials.

Introduction

MXene is a novel 2D nanomaterial discovered by a research group under the leadership of Professor Yury Gogotsi at Drexel University in 2011[1]. Many unique physical properties and electrochemical characteristics of MXene have been extensively studied due to its unique structure and composition (with transition metal and carbides/nitrides). Notably, MXene can form stable suspensions in water, has various broad absorption capabilities for electromagnetic waves, exceptional conductivity, and various surface terminations which are redox activated. These attributes render MXene suitable for fabricating pseudocapacitors capable of achieving high power density and energy density. It has versatile applications in electromagnetic wave interference shielding, energy storage, and optoelectronics, among others[2].

The unique physical and chemical properties of MXene provide new avenues for smart agriculture. Moreover, the biocompatibility of MXene with relatively simple plant systems is a prerequisite for further research into more intricate animal systems. On the other hand, with the potential for large-scale production of MXene in the future, whether plants can effectively absorb MXene's residues from water sources and soil is also worth exploring. Studies on the biocompatibility of MXene can also help us better observe the interactions between the surface functional groups of MXenes and the plant body, thereby deepening our understanding of MXene structures and properties. There have been many published studies on the impact of other nanomaterials on plant growth. For example, a study by Fang's group from the Beijing National Laboratory for Molecular Sciences in 2009 showed that carbon nanotubes could serve as transporters across plant cell walls[3]. Another example is the study by Sunho Park and others, which demonstrated that specially prepared graphene oxide promotes the growth of Arabidopsis thaliana and watermelon[4]. In 2022, Hongwei Zhu and other researchers provided insights into how graphene influences certain types of enzymes in plant roots, therefore affecting plant root growth[5]. However, there are limited studies on the biocompatibility of MXene, especially its compatibility with plants. Research conducted by Yan. et al suggests that MXene can adsorb and encapsulate lead ions in the soil, thereby preventing the harmful absorption of lead ions and promoting plant growth[6]. However, the paper did not mention the absorption of MXene by plants, the distribution of absorbed MXene within plants, and the impact of MXene on plant growth.

This work mainly focuses on the role of MXene in the growth of soil-grown and hydroponic plants. This involves a comparative analysis of plants cultivated in soil, supplemented with Ti_3C_2 suspension, with plants grown hydroponically in Ti_3C_2 suspension, juxtaposed against plants grown under standard conditions. Ti_3C_2 is a prominent representative within the MXene category, characterized by its moderate flake size and relatively straightforward preparation method. Notably, Ti_3C_2 exhibits room-temperature stability and commendable photostability, attributes of utmost significance given the pivotal role of sunlight in facilitating plant growth. Furthermore, Aliana Kanbata Pendias, in her work 'Trace Elements in Soils and Plants'[7], posits that titanium concentrations below 50 mg/kg generally do not exert toxic effects on plants. Moreover, a study published in 1939 by D. I. Arnon and P. R. Stout[8] suggests that while titanium is not essential for plant growth, it can, at lower concentrations, exert a stimulatory effect[9]. In summary, Ti_3C_2 emerges as an ideal material for conducting biocompatibility experiments with plants.

Materials and Methods

MXene Preparation and Delamination

Ti_3C_2 was prepared by selective etching from the MAX phase Ti_3AlC_2 under normal guidelines[10]. Delamination is then needed to make smaller flakes, which are easier for plants to absorb. According to previous research, Li^+ which is commonly used for MXene delamination is harmful to the plant body[11]. Thus additional centrifuge processes are needed to remove the residue Li ions in the MXene layers. 2.00 g of Ti_3C_2 was mixed with 1.42 g LiCl in 100 g H_2O. The mixed liquid was run under ultrasonic for 30 minutes. Then the liquid was centrifuged at 10, 000 rpm and 20 minutes, the supernatant was collected, and the sediment was refilled to 100 mL, thoroughly shaken, then centrifuged again. The whole process was repeated 7 times. and the solution washed and condensed theTi_3C_2 to make sure the suspension was mainly single-layer MXene with shallow Li^+ concentration so that it would be tolerable for the plant.

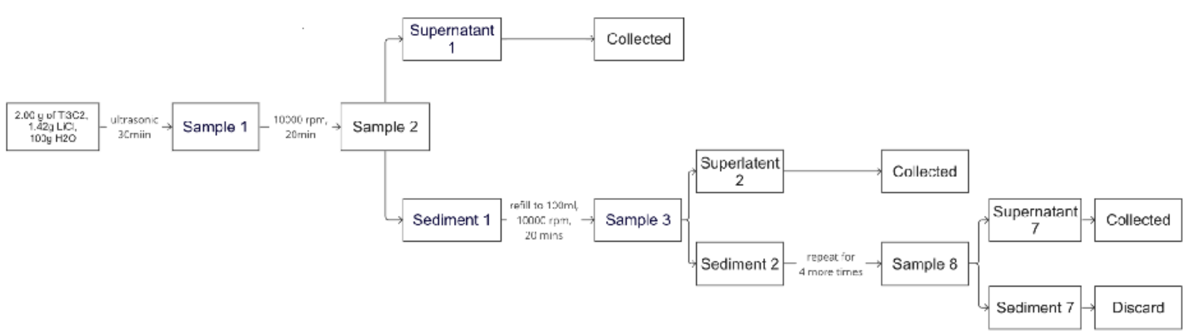

Figure 1. A flow chart of the delamination process

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The particle size of the delaminated Ti_3C_2 was checked by utilizing Dynamic Light Scattering (DLS) analysis. According to preliminary research done by Wang, et al., nanoparticles with a size less than 1 micrometer in diameter could be taken into the cell by endocytosis[12]. As shown in the figure below, the DLS indicated that a significant portion of the flake size is under 1 micrometer which means they could be taken in by the plant.

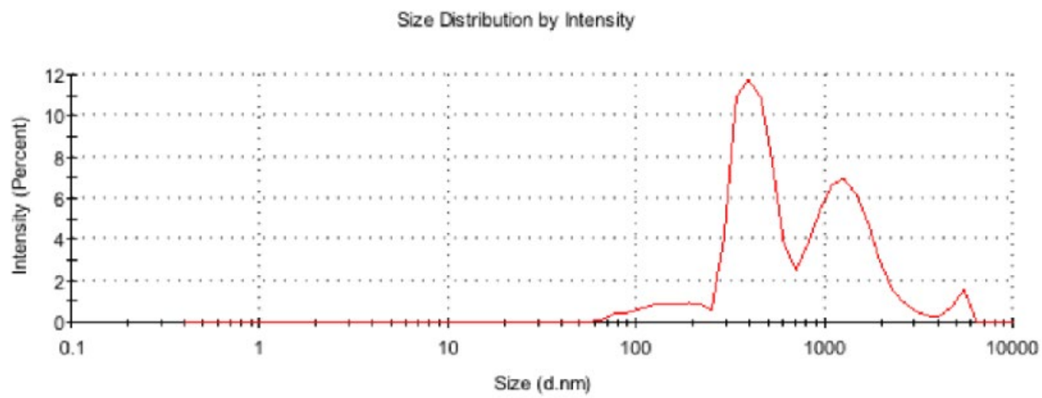

Figure 2. The particle size distribution we get from a DLS test of the Ti_3C_2 suspension we collected.
Subsequently, the concentration of the final MXene suspension was measured by the drop-casting method. 0.3 mL of suspension was dried on a glass slide, the film was taken off and the mass of that film was measured. In 0.3 mL of Ti_3C_2 suspension, there was 7.2×10-4 g of Ti_3C_2. The concentration is thus reported to be 2.4 mg/mL.

Experimental Setup: The Soil-Based Group

Following an extensive review of the literature, Crossandra infundibuliformis, commonly known as the Firecracker Flower, was selected as the subject for the soil-grown plant group. This selection was based on documented evidence indicating its high water absorption rate, suitability for container growth, and optimal temperature range of 70-75 degrees Fahrenheit[13]. Based on this research, a daily watering volume of 100 mL was established for the experiment.

Subsequently, the plants were divided into five distinct groups by the research team.

Control group: 100 mL of deionized water per day was administered

Group 1: 100 mL of 0.05 mg/mL Ti_3C_2 suspension per day was administered

Group 2: 100 mL of 0.025 mg/mL Ti_3C_2 suspension per day was administered

Group 3: 100 mL of 0.01 mg/mL Ti_3C_2 suspension per day was administered

Group 4: 100 mL of 0.05 mg/mL Ti_3C_2 suspension was sprayed on per day

The concentration of the Ti_3C_2 suspension applied to each plant was determined by referencing the concentration of graphene oxide utilized in a study on watermelons[4], conducted by Sunho Park and colleagues.

Figure 3. The firecracker(left) and the water hyacinth (right)

Experimental Setup: The Water-Based Group

Water hyacinth (Eichhornia crassipes) was selected as the experimental subject for the water-based group due to its notable water absorbency, rapid growth rate, and proven resilience in heavily polluted environments[14]. Initially, tap water was utilized as the cultivation medium, based on the hypothesis that essential salts required for plant growth may be contained within it.

However, on a subsequent day, MXene deposition was observed across all experimental groups. This phenomenon was hypothesized to occur as a result of the propensity of MXene flakes to attach to free ions in the tap water. This is likely attributable to the negatively charged surface groups of MXene, such as F- and OH-, which are believed to have an affinity for binding with positively charged metal cations, including Ca^{2+} and Mg2+.

In response to this observation, the cultivation medium was altered on the second day of the experiment. Ti_3C_2 suspensions in deionized water were introduced as the new cultivation base for all groups, with one group being retained as a control, continuing to use tap water.

The experimental groups were thus established as follows:
Control: 2 L of deionized water
Plant 1: 2 L of 0.01 mg/mL Ti_3C_2 suspended in deionized water
Plant 2: 2 L of 0.001 mg/mL Ti_3C_2 suspended in deionized water
Plant 3: 2 L of 0.0001 mg/mL Ti_3C_2 suspended in deionized water
Plant 4: 2 L of 0.01 mg/mL Ti_3C_2 suspended in tap water

The concentrations of Ti_3C_2 employed in this study were determined based on the graphene oxide concentrations used by Sunho Park and colleagues in previous research. To compensate for potential nutrient deficiencies associated with the use of deionized water as a cultivation base, a 12-4-8 (N-P-K) fertilizer was administered to all experimental groups.

The water hyacinth samples designated for Scanning Electron Microscopy (hereinafter written as SEM) and Energy Dispersive Spectroscopy (hereinafter written as EDS) analyses were initially frozen in a refrigerator. Subsequently, they were subjected to freeze-drying in a liquid nitrogen freeze dryer for 20 hours before examination.

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Results and Analysis

The Study of Ti_3C_2's Biocompatibility Using the Soil-Based Firecracker Flowers

The Growth of Crossandra infundibuliformis Might Experience a Certain Level of Inhibition in the Presence of Ti_3C_2 Suspensions

Over a twenty-day tracking period, the stem length and overall height of the Crossandra infundibuliformis plants, commonly known as Firecracker, were systematically monitored. The stem length was defined as the measurement from the point where the longest stem of the plant emerges from the soil to its tip. The plant height was quantified as the vertical distance from the lowest point of the soil to the highest point of the plant, measured in a stable plane without external support. Data were collected daily at 17:00 and were used to construct the subsequent charts.

Analysis of the data in Fig. 4, in conjunction with photographic records of the plant growth conditions, indicates that a significant adverse effect on the viability of the Firecracker plants was not exerted by the MXene solution. Throughout the monitoring period, a consistent upward trend in both height and stem length was demonstrated by all plants.

However, given the inherent complexity of soil systems, it is plausible that the Ti_3C_2 solution was not substantially absorbed by the Firecracker plants. As such, definitive conclusions regarding the biocompatibility of Ti_3C_2 with Firecracker plants remain to be established.

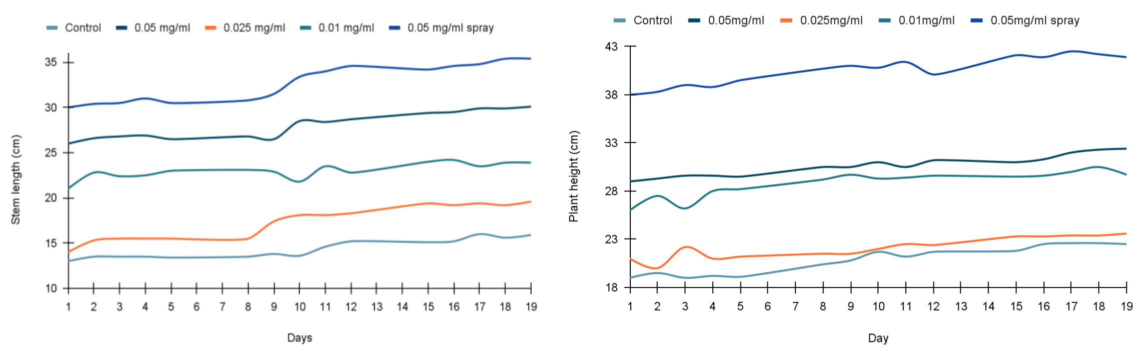

Figure 4. The stem length (left) and plant height of the plant (right) with different amounts of Ti_3C_2 over the tracking period.

Subsequently, the percentage growth in stem length and plant height relative to their initial values for the Firecracker Flower (Crossandra infundibuliformis) plants was calculated and graphically represented, as depicted in Figure 5. A discernible relationship with the concentrations of the Ti_3C_2 suspensions used was not exhibited by the growth in stem length. A decreasing trend as the concentration of the Ti_3C_2 suspensions increased was demonstrated by the percentage growth in plant height relative to the initial height. This trend was especially notable in plants that were treated with the Ti_3C_2 suspension via spray application, where the growth as a percentage of the original height was found to be minimal.

However, it is noted that the plant height is not a parameter that is as stable as the length of the longest stem. For instance, an apparent increase in measured height could potentially be caused by soil erosion resulting from watering, and the recorded height could also be affected by the plant's position on a given day. Consequently, whether MXene exerts an inhibitory effect on the growth of plants cannot be definitively ascertained.

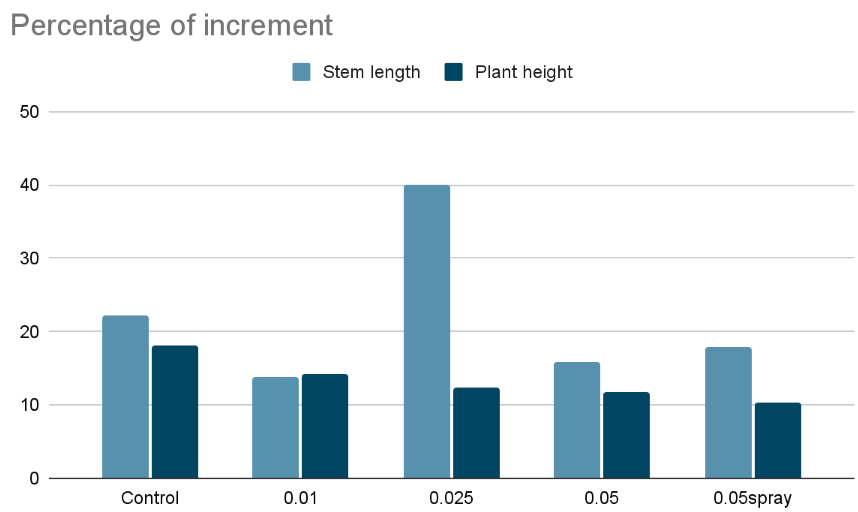

Figure 5. The increment of stem length and plant height in the percentage of their original value.

The Elliptical Structures Discovered are Hypothesized to be Inherent to the Leaves of the Firecracker Plant

As depicted in Figure 6, the images are represented as optical microscope photographs of the plants in Group 3. It is noted that in all-optical microscope images used in this study, 1 cm in the image approximately corresponds to 150 micrometers in the real world. The left image is presented as an optical micrograph of the leaf surface, where numerous black spots were observed. These black spots were also identified in the middle image, allowing the preliminary conclusion that they are not attributable to Ti_3C_2 to be drawn. The nature of these black spots remains undetermined at this stage, and further investigation using SEM may be warranted.

When the left and middle images are compared, it is apparent that the left image is darker, and black impurities in the superficial cell walls of the leaves are suspected to be Ti3C2. However, to confirm whether these impurities are indeed MXene-derived, further analysis using EDS or SEM is deemed necessary. The observed differences may merely be the result of variations in lighting conditions during microscopic imaging.

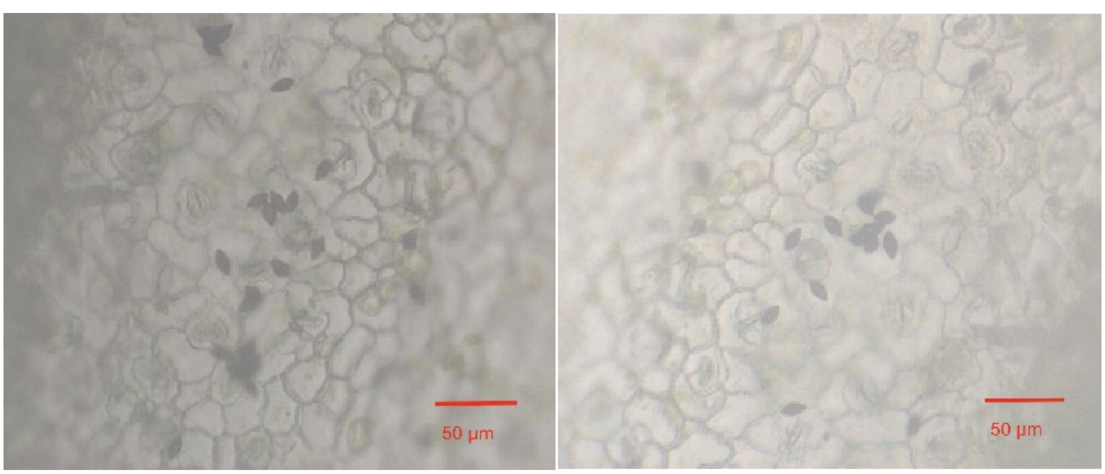

Figure 6. The microscopic image of group 3 (0.01 mg/mL Ti_3C_2) plant's leaf surface (left), and control group leaf surface(right)

The Presence of Nematodes in the Soil Further Provides Evidence for the Soil System's Adaptability to Ti_3C_2

In Figure 7 (right), transparent organisms were observed, and after comparison with relevant literature, they were identified as a certain type of nematode. These nematodes were found to be present in the soil samples of each plant group and were consistently observed in the topsoil layer. This observation suggests that a degree of resistance or biocompatibility with Ti_3C_2 is exhibited by these nematodes. Notably, black particles were observed in the midsection of the nematodes' bodies. The initial hypothesis was that these might be ingested MXene particles. However, when compared with the structure of the nematodes in the middle-right image, no black regions were revealed at the corresponding digestive system locations[15]. Thus, it is postulated that the black regions likely represent the nematodes' reproductive organs rather than MXene, although final confirmation will require EDS verification.

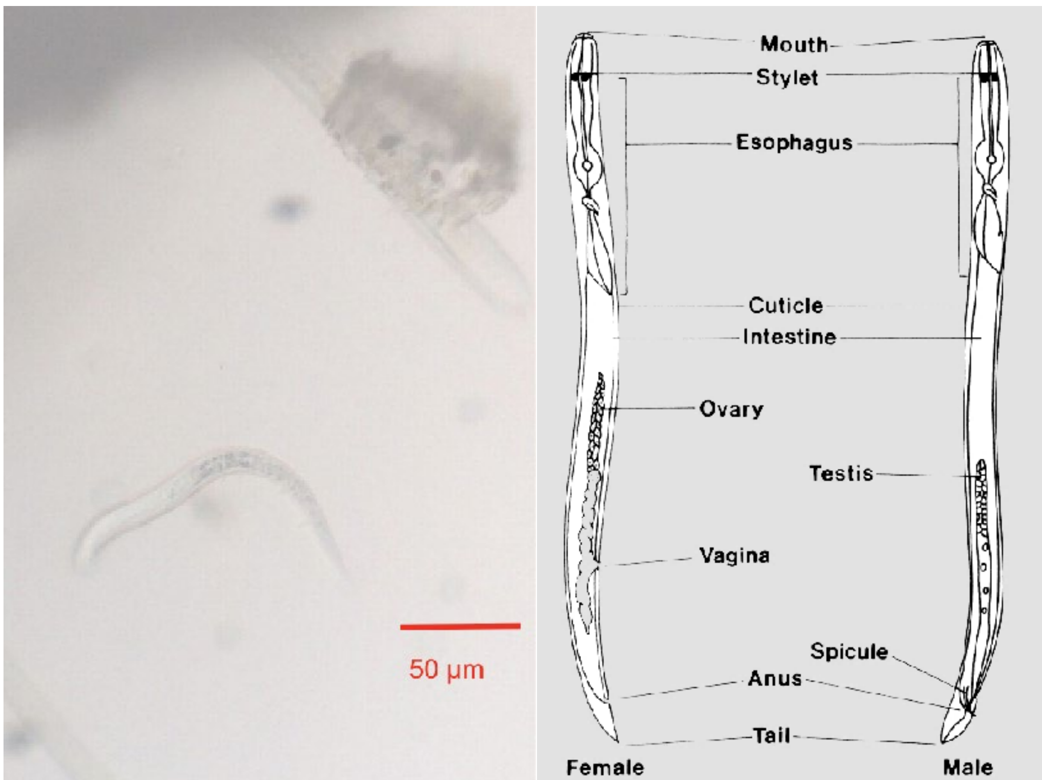

Figure 7. The nematode appeared in the group 3 soil sample (left) and a schematic shows the structure of a nematode(right)

As depicted in Fig. 8, a comparison reveals that notably darker regions at the epidermal cell walls are exhibited by the petals in the left image, which were treated with a Ti_3C_2 spray. However, due to the lack of three-dimensional observation capabilities, it is challenging to ascertain whether this darkening is due to Ti_3C_2 residues lodged within the grooves between the surface cells of the petals, or if Ti_3C_2 is more readily absorbed by the epidermal cell walls.

Interestingly, similar darkening was not observed on the leaves, which also have a textured surface and were treated with the Ti_3C_2 spray. Instead, a reflective effect was exhibited by unabsorbed MXene on the leaf surface. Based on these observations, it can be preliminarily inferred that the darker appearance of the petal epidermis is likely due to the absorption of Ti_3C_2 .

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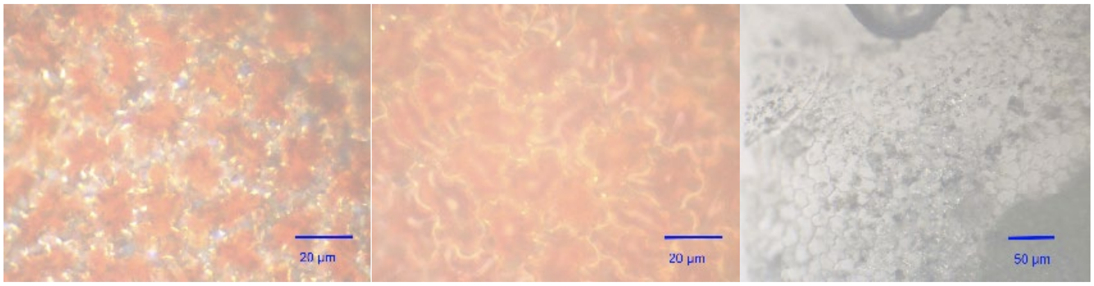

Figure 8. Microscopic picture of flower with MXene sprayed on (left), flower without MXene sprayed on (middle), and leaf surface with MXene sprayed on (right).

The Petal Surfaces of Plants Exhibit a Greater Affinity for Ti_3C_2 when Contrasted with Leaf Surfaces

Another intriguing observation that may indirectly substantiate this inference was made following the application of Ti_3C_2 spray to the surfaces of both flowers and leaves of the same plants. It was noted that two days post-application, virtually no discernible black spots were exhibited on the flower surfaces to the naked eye, while a visible reflective film was retained on the leaf surfaces.

It is hypothesized that this differential capacity for MXene absorption between the plant's flowers and leaves is attributable to the distinct surface structures of these organs. The leaf surface, designed for water retention, is exhibited to have lower permeability, whereas the flower surface is observed to possess a more extensive pilose structure and demonstrate higher permeability. This structural distinction likely underlies the common observation that flowers tend to wilt before leaves when a plant is subjected to water stress.

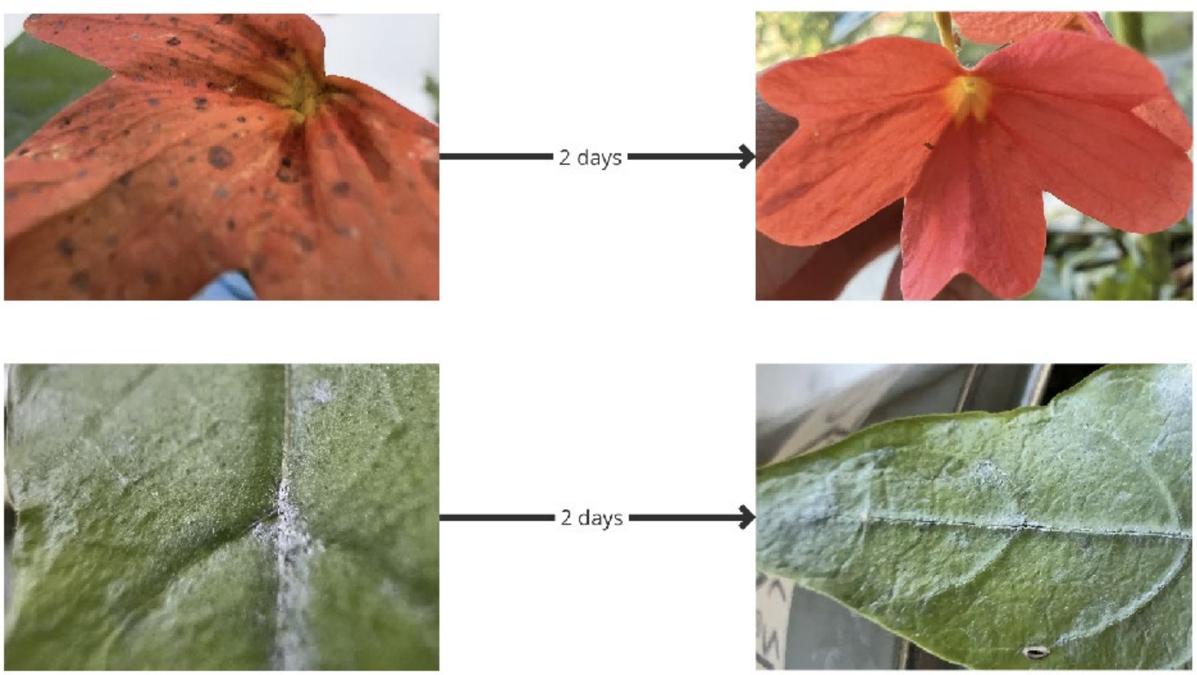

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Figure 9. Flower on the day MXene sprayed on (top left); Same flower two days after MXene sprayed on (bottom right); Leaf on the day MXene sprayed on (top right); Leaf two days after MXene sprayed on (bottom right).

The Study of Ti_3C_2's Biocompatibility Using the Water-Based Water Hyacinth

Higher Concentrations of Ti_3C_2 in the Suspension Correlate with a Greater Proliferation of Water Hyacinth Roots

Systematic tracking of various parameters of the water hyacinth plants, including the maximum leaf width, the longest root length, the number of roots, and the number of leaves, was conducted. Upon comprehensive analysis, it was observed that only the number of roots exhibited significant variation over time. The remaining parameters either remained relatively constant or did not effectively reflect the overall growth condition of the plants.

As depicted in Figure 10, the root count data for five water hyacinth specimens over the tracking period was compiled and analyzed. Both the absolute increase in root number and the percentage increase relative to the initial root count were compared. Notably, it was observed that water hyacinth plants grown in higher concentrations of Ti_3C_2 suspensions exhibited greater increments in root count.

A plausible explanation for this observation is that MXene flakes may obstruct the sites at the plant roots where nutrients are absorbed. To compensate for the reduced nutrient uptake due to these obstructions, additional roots may be proliferated by the plants to offset the regions blocked by MXene flakes.

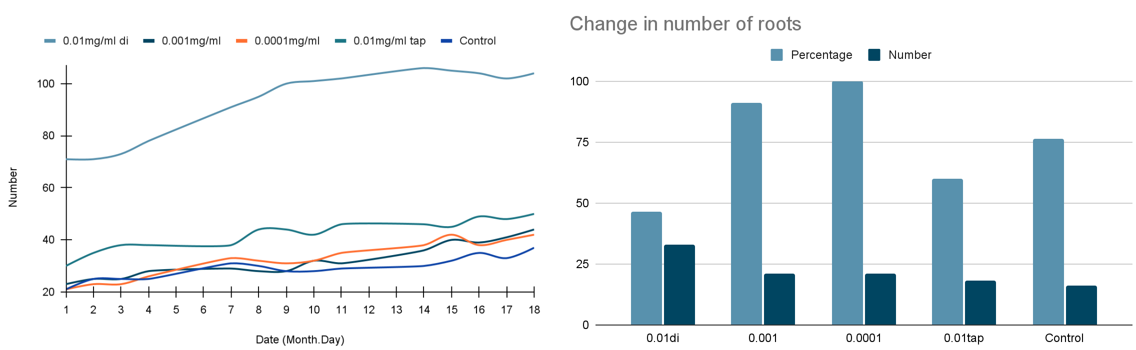

Figure 10. The number of roots over the tracking period (left); The change in the number of roots in number and percentage of the original value (right).

In addition, as illustrated in Figure 11, it is observed that with the increasing concentration of Ti_3C_2 suspensions, the root fluff of the water hyacinth plants becomes thicker, longer, and darker in color. The darkening is highly likely to be attributable to the absorption of MXene, a hypothesis that was further substantiated in subsequent analyses. The increase in thickness and length of the root fluff is likely to be driven by a mechanism similar to that which triggers the proliferation of additional roots, serving to enhance the plant's capacity for nutrient uptake under these conditions.

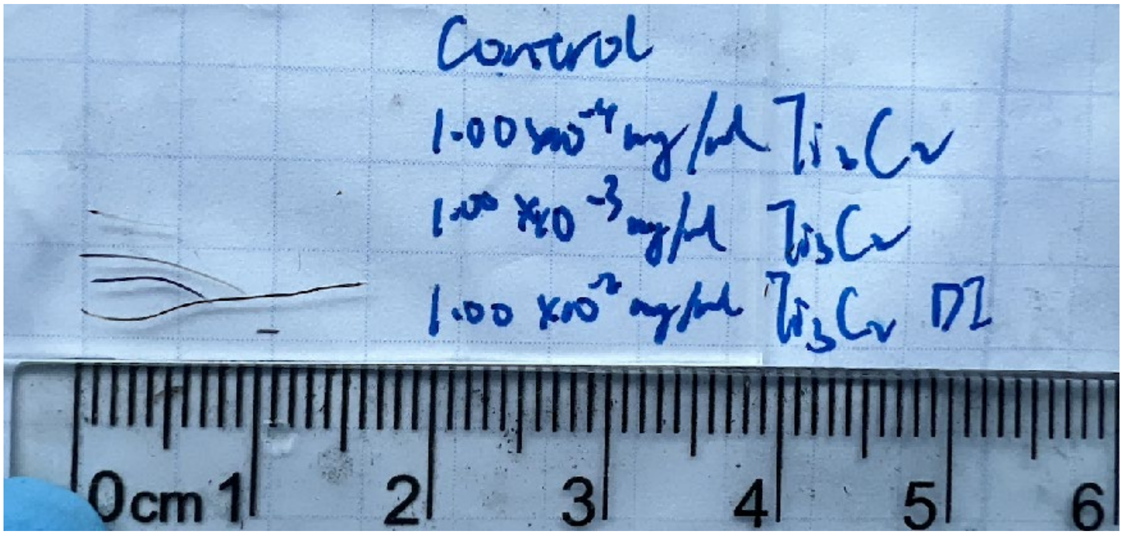

Figure 11. A comparison of the morphology of the root fluffs of the four plants

Ti_3C_2 is Absorbed by the Roots of Water Hyacinth but Does Not Appear to Translocate to the Upper Structures of the Plant.

Subsequent analyses were conducted using EDS on the root fluff of Plant 1 (0.01 mg/mL Ti_3C_2), the stem bulb of Plant 1, and the root fluff of a control plant. According to the EDS results presented in Figure 12, a distinct titanium signal was detected exclusively in the root fluff of Plant 1. In light of the absence of a titanium signal in the control group and the low likelihood of alternative sources of titanium ions in the plant's growing stem, the titanium signal in the root fluff of Plant 1 can be confidently attributed to the presence of Ti_3C_2 . The absence of a titanium signal in the bulb of Plant 1 further suggests that penetration of Ti_3C_2 into the upper structures of the water hyacinth plants did not occur.

However, the stage at which the upward translocation of Ti_3C_2 is halted cannot be definitively ascertained. More detailed cross-sectional EDS analyses will be necessary in future research to further investigate this issue.

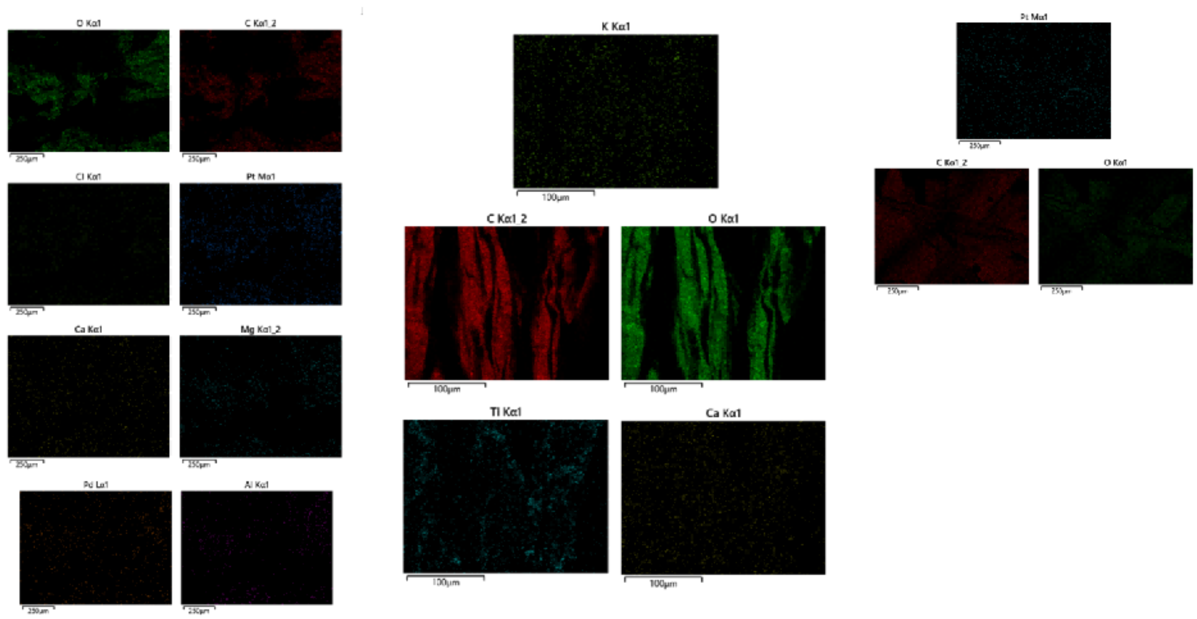

Figure 12. The EDS result of the bulb of plant 1 (left), the root fluff of plant 1 (middle), and the root of the control group plant (right).

Ti_3C_2 Exposure is Associated with Observable Structural Damage to the Root Hairs of the Plants

A comparative analysis of SEM images of root hairs from the control group (right) and the 0.01 mg/mL Ti_3C_2 group (left) is presented in Figure 14, with images captured at similar magnification levels. It is observed that the surface of the root hairs of water hyacinth plants grown in the MXene solution appears markedly rougher. It is hypothesized that this coarser appearance may be attributed to damaged surface cells, and the adherent flake-like structures are likely identified as Ti_3C_2 flakes.

Initially, it was considered that the epidermal cells depicted in the lower left image might have been damaged during the freeze-drying process. However, upon further examination, it was observed that this rough surface texture was consistently present in plants cultivated in the 0.01 mg/mL Ti_3C_2 suspension but was absent in the control plants. This observation largely negates the hypothesis that the damage was caused by the freeze-drying process, suggesting that the observed damage to the surface cells of the plants is likely attributable to Ti_3C_2 .

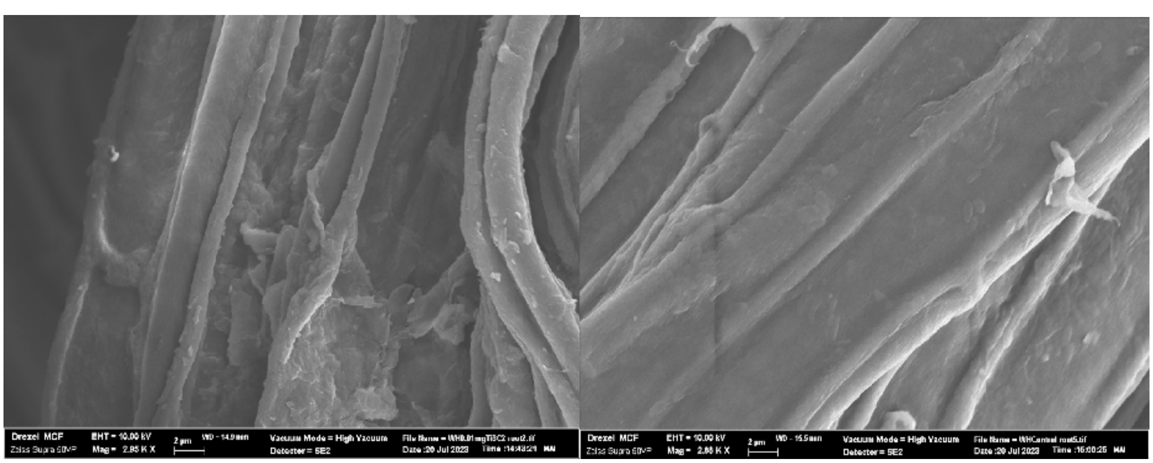

Figure 13. The SEM image of the root fluff of water hyacinth plant 1 (left) and control group water hyacinth (right) under approximately the same magnification.

As depicted in Figure 15, SEM images of the root hairs of plants exposed to a 0.01 mg/mL Ti_3C_2 solution are presented. Notable adherence of Ti_3C_2flakes to the root hairs can be observed. These flakes, which have not been fully delaminated, are presumed to be too large to be absorbed by the plant due to their size. Through the magnified view provided in the right-hand window of the figure, it can be seen that the Ti_3C_2 flakes are closely adhered to the surface of the plant tissues. From this, it is inferred that some form of interaction has likely occurred between the surface of the MXene and the surface of the root hairs.

Large MXene Flakes were Found to Adhere Tightly to Plant Cell Surfaces

The surface groups of Ti_3C_2 are predominantly composed of OH- and a small quantity of F-, both of which are negatively charged groups. The surface groups of plant roots are typically composed of -COOH, -OH, -NH2, and -H2PO4[16]. It is suggested that the surface groups of MXene may have undergone organic reactions (such as dehydration, addition, substitution, etc.) with the surface groups of the plant roots, resulting in their adherence.

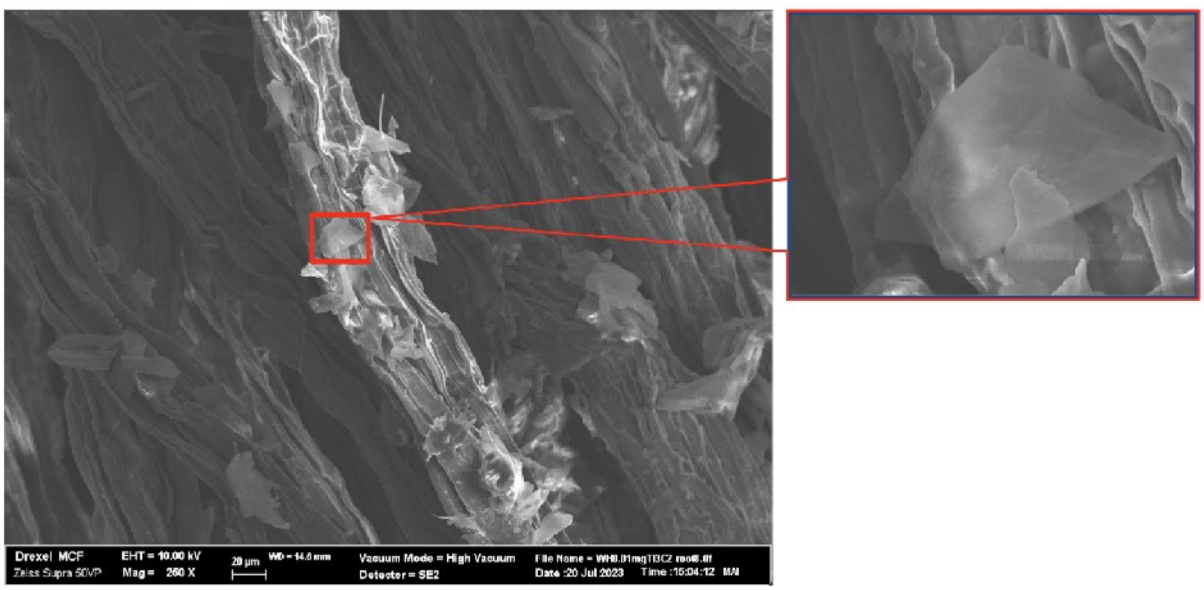

Figure 14. The SEM image of water hyacinth plant 1's (0.01 mg/mL Ti_3C_2) root fluff and a magnification of one MXene flake.

In Figure 16, a greening of the culture medium is observed in the plant grown in deionized water around day 19. Through investigation, this greening is believed to be attributable to a form of algal bloom, a phenomenon that is also manifested in Plant 2, which was exposed to a lower concentration of Ti_3C_2 suspension. However, this phenomenon is not observed in Plant 1, which was exposed to a higher concentration of Ti_3C_2 suspension. This observation is taken as evidence that a certain inhibitory effect on algae is exerted by MXene.

Furthermore, it is noted that even when deionized water is used to prepare the MXene suspension, deposition of MXene flakes still occurs after a certain period of time. Two potential explanations for this phenomenon are proposed: First, it is possible that a reverse osmosis phenomenon, triggered by the ionic concentration of the suspension, could have led to the efflux of ions from the plant tissues. Alternatively, the dispersion of titanium ions within the solution might have resulted from the photodegradation of Ti_3C_2 . The presence of cations is thought to cause the negatively charged surface groups of Ti_3C_2 to preferentially envelope these cations, thereby leading to the deposition of Ti_3C_2.

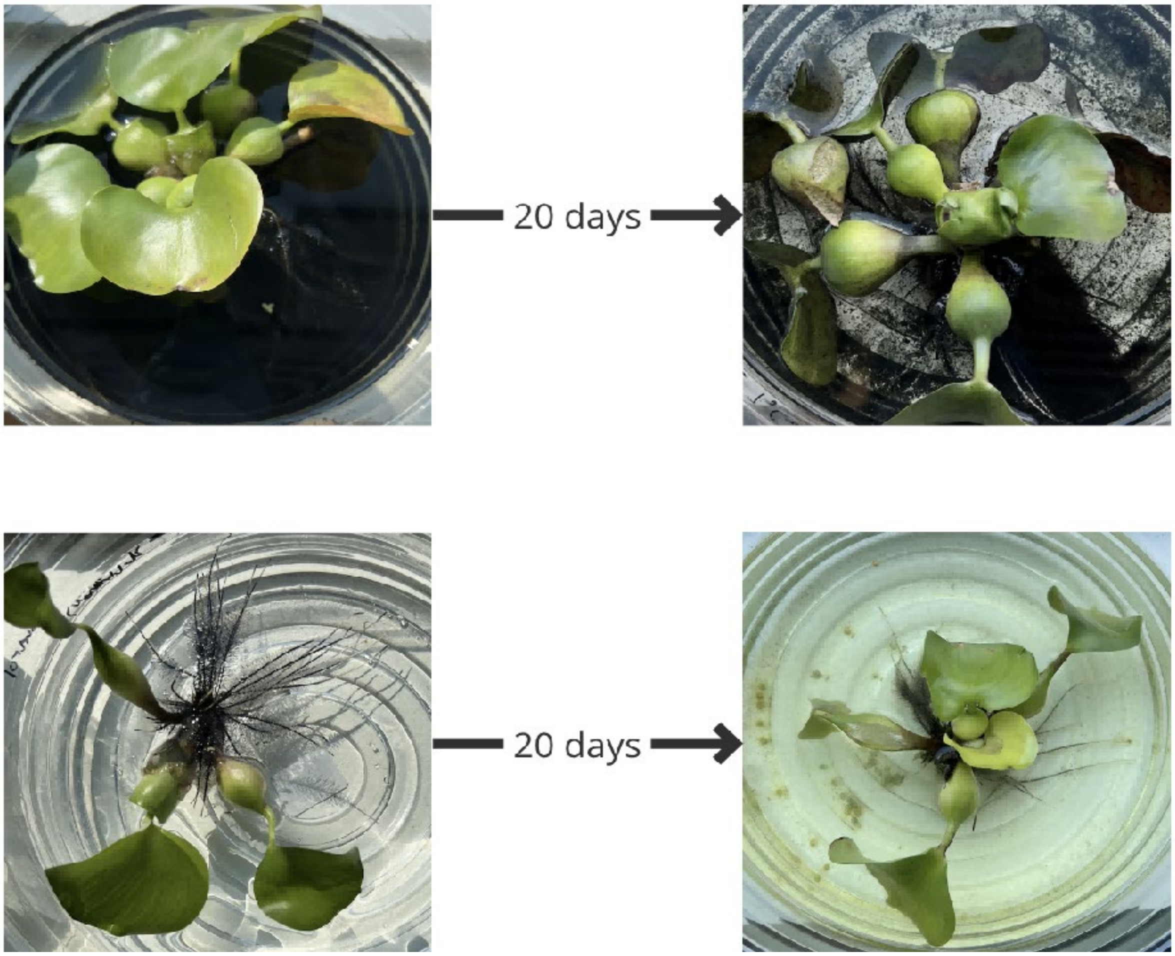

Figure 15. A comparison of water hyacinth that grew in 0.01 mg/mL Ti_3C_2(top) and deionized water, (bottom) at the beginning and the end of the 19 days tracking period.

Conclusion

In summary, the available evidence implies that Ti_3C_2 showcases favorable biocompatibility within intricate soil systems. This phenomenon could potentially stem from the inherent self-regulation mechanisms within these systems, or from the influence of soil constituents on the interplay between Ti_3C_2 and plant roots. The data suggests that the growth of Crossandra infundibuliformis, commonly referred to as the Firecracker Flower, might experience a certain level of inhibition in the presence of Ti_3C_2 suspensions. Nonetheless, owing to the absence of consistent trends across various metrics, a conclusive inference cannot be definitively made at this juncture. After analyzing the optical microscope images of Firecracker Flowers, we discovered black elliptical structures in the leaves and nematode larvae in the soil. Upon further investigation, we concluded that the elliptical structures are inherent to the leaves of the Firecracker plant. The presence of nematodes in the soil further provides evidence for the soil system's adaptability to Ti_3C_2. The experimental outcomes also indicate that the petal surfaces of plants exhibit a greater affinity for Ti_3C_2 when contrasted with leaf surfaces, leading to a discernible accumulation of Ti_3C_2 at cell walls. Additionally, it is noteworthy that the introduction of MXene appears to exert negligible effects on the survival of nematodes within the soil environment.

In hydroponic systems, which are inherently less complex than soil systems, a notable deterioration in plant health was observed after 20 days of exposure to MXene suspensions, as evidenced by Figure 15. The data suggests that higher concentrations of Ti_3C_2in the suspension correlate with a greater proliferation of water hyacinth roots. This is postulated to be a compensatory response by the plants to ensure adequate nutrient uptake in the presence of MXene, which may obstruct normal nutrient absorption. This response is analogous to the behavior of water hyacinth in polluted water conditions, suggesting that MXene may be recognized by water hyacinth as a pollutant to some extent[17]. The data confirms that Ti_3C_2 is absorbed by the roots of water hyacinth, but does not appear to translocate to the upper structures of the plant. Moreover, Ti_3C_2 exposure is associated with observable structural damage to the root hairs of the plants, resulting in coarser surface morphology. Unabsorbed, larger MXene flakes, likely due to interactions with surface groups, were found to adhere tightly to plant cell surfaces.

In conclusion, the preliminary findings of this study suggest that while Ti_3C_2 demonstrates a degree of biocompatibility, it is also likely to exert a mild negative impact on plant physiology. Ti_3C_2 appears to be recognized by plants as a hydrophilic impurity and may enter and exit plant cells via endocytosis and exocytosis. However, its diffusion within the plant body appears to be significantly restricted.

Future research directions proposed by this study include but are not limited to, investigating the response and absorption of carnivorous plants to MXene, conducting more detailed cross-sectional SEM and EDS analyses for terrestrial and aquatic plants to quantitatively study the diffusion of MXene within plant tissues, what structure of the plant stops MXene from diffusing and extending the monitoring period to assess the long-term effects of varying MXene suspension concentrations on plant growth.

Acknowledgments

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