Monitoring the Environmental Impact of TiO₂ Nanoparticles Using a Plant-Based Sensor Network

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Abstract—The increased manufacturing of nanoparticles for use in cosmetics, foods, and clothing necessitates the need for an effective system to monitor and evaluate the potential environmental impact of these nanoparticles. The goal of this research was to develop a plant-based sensor network for characterizing, monitoring, and understanding the environmental impact of TiO2 nanoparticles. The network consisted of potted Arabidopsis thaliana with a surrounding water supply, which was monitored by cameras attached to a laptop computer running a machine learning algorithm. Using the proposed plant sensor network, we were able to examine the toxicity of TiO₂ nanoparticles in two systems: algae and terrestrial plants. Increased terrestrial plant growth was observed upon introduction of the nanoparticles, whereas algal growth decreased significantly. The proposed system can be further automated for high-throughput screening of nanoparticle toxicity in the environment at multiple trophic levels. The proposed plant-based sensor network could be used for more accurate characterization of the environmental impact of nanomaterials.

Index Terms—Biosystems, environmental monitoring, nanobioscience, nanobiotechnology.

I. INTRODUCTION

ONCERNS about the impact of nanoparticles to environmental health and safety are rapidly increasing commensurate to their use in cosmetics, food, and paint industries [1]–[7]. Despite their widespread use, it is still unclear how these nanoparticles might impact the environment. Much research has focused on the effects of nanoparticles in mammalian systems, with few efforts examining the environmental impact in either the aquatic environment or on terrestrial plant

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species [8]–[11]. Specifically, most research has focused on the *in vitro* toxicity of metal nanoparticles to the lung, liver, brain, and skin [12]–[19]. Only recently have researchers begun to examine organic nanoparticles as an alternative to their metal-based counterparts [20]. Through the course of these studies, like metal nanoparticles, the toxicity of the organic nanoparticles has been found to depend on their structures and bioavailability [21]–[26]. While these studies are crucial for determining the direct impact on human and mammalian health, it does not address the issue of environmental toxicity, or develop a method for determining the level of nanoparticle exposure in the environment.

There is an increasing need to develop a high-throughput, reliable system for monitoring the environmental impact of nanoparticles at various levels. However, significant difficulties exist in the development of a procedure to determine if an environment has been exposed to potentially hazardous nanoparticles. Often the use of large and expensive equipment, such as aerosal-mass spectrometry systems, is necessary to detect nanoparticle exposure [27]. Other options include delayed detection through inductively coupled plasma atomic emission spectroscopy, size-exclusion chromatography, microfiltration, field-flow fractionation, and capillary electrophoresis [28]. These detection systems have several disadvantages that make them impractical to use for broad landscape-level environmental monitoring: 1) detection cannot be conducted at the potential source of exposure; 2) the use of these techniques is relatively costly and time-consuming; 3) detection requires an experienced operator to conduct the testing; and finally, 4) even if the systems detect nanoparticles in the environment, they are not capable of determining if the nanoparticles have a negative effect on the ecosystem. In an effort to develop a more effective sensor for environmental monitoring of the effects of nanoparticles, we propose a sensor network-based approach in this paper. The purpose of the sensor network is to broadly interrogate biomes for potential hazards.

Sensor networks have gained broad attention in recent years with their ability to measure and gather information from the environment, and, based on local decision-making processes, transmit the data to a user. With the advent of the internet and smart phones, the applicability of these systems has rapidly been embedded into our daily lives. These systems may acquire data continuously and transfer high-fidelity data across a network. As a result, the sensor nodes should strive to process the raw sensor signal locally and perform local decision making to determine the most "interesting" signals/events, such as detecting anomalous events. Local processing and decision making avoids

wasting resources on "uninteresting" data, such as sending normal raw sensor readings to a human operator for interpretation, which could be regarded as noise. Distributed decision-making algorithms for anomaly detection in natural environment greatly simplify the jobs of human operators. In anomaly detection applications, a wireless sensor node in the network can monitor its local region, and communicate through a wireless channel with other nodes to collaboratively produce a high-level representation of the environment. Using such a network, a large area can be monitored at a relatively low cost. The challenge for implementing the sensor network lies in the fundamental understanding of the sensors, modeling of the sensor data, and adherence to appropriate engineering principles. Data modeling in such distributed sensor networks is critically important for determining the "normal" operation mode of the system. This will allow the system to perform autonomous anomaly detection to recognize when unexpected sensor signals are detected. The advantage of this approach is that the designer does not have to characterize the anomalous signatures in advance. Instead, the system of sensor nodes can learn this characterization autonomously, for application to new domains. For environmental monitoring of the effects of nanoparticles, we have chosen to build a sensor network using biological system sensors that can be easily embedded in the environment.

Instead of deploying costly hardware, biological sensors such as plants and algae are capable of detecting chemical and physical toxins in the environment at relatively low levels, extending the "vision" of the sensor network, and integrating signals [29]. Plant-based biosensors ("phytosensors") have previously been used to detect environmental exposure from a variety of contaminants, including radiation, heavy metals, and chemical toxins [30], [31]. Many of these systems use microarray studies to determine genes that are up regulated by a specific toxin. Once identifying the genes, a fluorescence protein (FP) reporter gene can be coupled to the gene or promoter of interest and the expression of the gene can then be monitored externally. This kind of transgenic biosensor requires a significant amount of time and resources to develop, and is generally highly specific to a certain toxin. This approach has led to the development of very specific biosensors, but not one that can determine exposure from a wide range of toxins—a generalist sensor that acts as a "check engine light." To circumvent this problem, we have developed a phytosensor that is capable of detecting the environmental impact of nanomaterials on plants using a variety of cues to reflect plant health. At the early stages, this system can be used to identify plants that are sensitive to the nanoparticles before costly FP-based transgenic systems are developed. These generalist phytosensors have the advantage of being noninvasive, directly demonstrate positive and negative responses from the biosensors, and are cost effective. A network of biosensors has great potential for applications, such as environmental monitoring [32], water quality monitoring [33], and health monitoring [34]. Since plants have not previously been used as sensors to monitor the environmental impact of nanoparticles, and differences may occur between different plant species, online learning algorithms are desirable to develop and employ for the system.

TABLE I KNOWN NANOMATERIAL TOXICITY IN Arabidopsis

Nanoparticle	Conc. (mg/l)	Time (days)	Toxicity level	Ref.
Al ₂ O ₃ , 150 nm	4000	18	25%	[37]
SiO ₂ , 42.8 nm	0-4000	18	None	[37]
Fe ₃ O ₄ , 50 nm	2000	18	20%	[37]
ZnO, 44.4 nm	400	18	90%	[37]
Single Walled Carbon Nano Tubes (SWCNT)	250	0.25	25%	[38]

For the initial testing, *Arabidopsis thaliana* has been used as the biosensor. This species has been chosen for the following reasons: 1) previous studies have demonstrated that it is susceptible to a variety of metal nanoparticles, including ZnO, Al₂O₃, SiO₂, Fe₃O₄, and SWCNT's (see Table I); 2) it is fast growing and compact; 3) it is the most widely adopted model plant for research; and 4) it has clear morphological changes after toxic exposure that can be easily analyzed through image processing. In general, toxicity in plants can be detected by monitoring changes in the shape, texture, color, and orientation of the phytosensors. These factors have previously been proven effective at determining stress or toxicity induced by pathogens, toxins, and radiation [35], [36].

In this study, individual sensor nodes were established using A. thaliana as the phytosensor. An array of sensor nodes was then combined to create a phytosensor network. A learning algorithm was used to train the system to identify changes in the environment. Finally, the system was used to test if toxic effects could be observed from the administration of TiO_2 nanoparticles. The results from this study demonstrate the feasibility of using a phytosensor network to monitor the environmental impact of nanoparticles at multiple trophic levels. The design of this system is readily expandable to a large number of sensor nodes capable of monitoring a large geographic area. By using machine learning techniques, the system is also highly adaptable and provides a robust way to detect abnormal changes in a defined set of plants.

II. MATERIALS AND METHODS

A. Phytosensor Setup

Arabidopsis thaliana ecotype "Columbia" plants were grown from seeds. Plants were chosen at four days of growth, and potted individually in 6 cm² pots. The plants were then moved to an environmental growth chamber set at a constant temperature of 25.2 °C and humidity level of 80%. The environmental growth chamber used a 16–8 day–night cycle to simulate realistic lighting conditions in temperate summer. Three plants were placed in a Tupperware container and compromised a single sensor (experimental unit). The use of a separate Tupperware container for each sensor allowed the introduction of the control (tap water) and test solutions (TiO₂ nanoparticles) without the risk of cross contaminating the sensors.

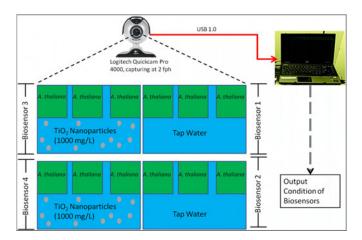


Fig. 1. Experimental platform. A biosensor is composed of three separately potted *Arabidopsis* plants exposed to the same environment containing either tap water, or tap water mixed with 1000 mg/ml TiO₂ nanoparticles. Images of all four biosensors are collected every 30 min by the camera and exported to the laptop through a USB 1.0 connection. Every two days 250 ml of tap water and fresh TiO₂ solution were added to the respective containers to maintain hydration.

B. Establishment of the Sensor Nodes

A complete sensor node consisted of phytosensor units, as defined earlier, an RGB camera, and a computer to analyze the acquired images (see Fig. 1). Logitech QuickCam Pro4000 cameras were used to monitor the biosensors and capture images for subsequent analysis. This camera is an off-the-shelf commercial product that provides photo quality even at low light levels. Additionally, since it is inexpensive, a large number of cameras can be easily deployed into the environment. The cameras were triggered, by the attached laptop computer, every half hour for one month to constantly determine the health of the plant (sensor). In the preliminary setup, the cameras were connected to the laptop through a USB connection; however, wireless data transfer could be used instead of USB transmission in future studies. A crucial step in establishing this type of sensor node is translating the image data into a measurement of plant health.

Typically, the key metrics used to determine the health of the plant include: shape and orientation of the leaves (an indicator of wilting), spectral characteristics of the leaf (color and temperature, indicators of photosynthesis), and the texture of the leaf (an indicator of the water content). In this study, we have chosen to focus only on the spectral characteristics associated with leaf color as a measure of the plant health. Previous studies have demonstrated the potential for machine vision to be used in the detection of plant toxicity due to plant pathogens, fungi, etc., [39].

The machine learning algorithm used in this study focused on comparison of the current captured image with that of the image captured at the previous time point. If the difference between the two pictures exceeded a threshold, then an abnormal change reading was detected. In addition, the picture frames were analyzed over time, to detect sequential anomalies associated with normal plant behavior associated with growth. To assess the color changes of the leaf, we utilized a histogram thresholding technique. First, the RGB image was converted to a grayscale

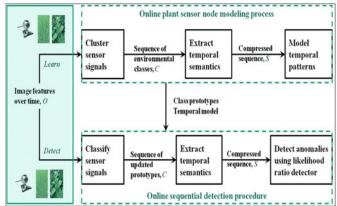


Fig. 2. Machine learning framework used for the biosensors. The top portion of the figure diagrams the learning procedure, whereas the bottom portion illustrates the detection procedure.

intensity image. The conversion eliminates the hue and saturation information while retaining the luminance. The algorithm converts RGB values to grayscale values by forming a weighted sum of the R, G, and B components. The weights for R, G, and B components were 0.2989, 0.5870 and 0.1140, respectively. After converting the images, they were segmented based on the distribution of "green" pixels. Finally, the segmented images were used to train the fuzzy adaptive resonance theory (ART) neural network.

C. Training of the Sensor Nodes

Fig. 2 shows the proposed learning and sequential detection procedures on a single sensor node. The first step in training is to determine the baseline values for the phytosensors by growing them in the optimal conditions in the environmental growth chamber. This allows the sensor nodes to develop a classifier for normal and expected signals. In addition to the classifier, a time analysis model was built to represent dynamic changes over the course of time. First, a likelihood-ratio sequential detection was used to detect anomalies. Next, the system used a classifier to categorize the stress cues into different environmental states and perform sensor fusion, using the fuzzy ART neural network [40]. The fuzzy ART system is an unsupervised artificial neural network that can perform dimensionality reduction and pattern classification simultaneously. There is no offline training phase required by the fuzzy ART neural network. The learned class prototypes can be updated at each sampling point. Therefore, it is adaptive to changes in both the environment and the phytosensors themselves. Moreover, the algorithm is simple enough to run in real time, while maintaining an acceptable performance level. Fuzzy ART was first implemented in the wireless sensor network [41], and has since been updated to improve anomaly detection performance by incorporating temporal and spatial information [40], [42], [43]. Being able to model and detect time-related changes allows periodic detection of the leaf color change, indicating exposure to the nanoparticles. In order to accomplish the detection of abnormal leaf color, we propose a three-step detection scheme. First, the semantics were extracted out of the temporal sequence using a symbol

compression technique called Lempel–Ziv–Welch [44]. Next, a variable memory length Markov model (VMM), i.e., a probabilistic suffix tree (PST) [45], was used to model the compressed temporal sequence. A variable memory Markov model preserves the minimal subsequences (of variable lengths) that are necessary for precise modeling of the given statistical source, resulting in a more flexible and efficient sequence representation than a traditional Markov model [46]. Finally, a universal background model (UBM) [47] likelihood-ratio detector was used to detect anomalies in the time patterns.

Phytosensors may have long periods of inactivity, i.e., nondetection, which would be computationally costly to model using the Markov-based time model alone. To avoid this expense, a symbol compression technique was used to map these temporal events/sequences into semantic symbols, prior to analysis with the VMM model. The advantages of the current model, over continuous-measuring models are: 1) digital image data are often discrete; 2) environments that are modeled with discrete states have clear physical interpretations and are therefore natural and easy for humans to interpret (e.g., toxic or nontoxic); 3) data compression techniques, which the system uses to reduce the size of the event sequence, typically require discrete state representations. To detect time-related changes, ideally, the system would build a normal life span of healthy plants and an abnormal lifespan after the plants have been exposed to the nanoparticles. In reality, it is difficult to model all different types of abnormal events, since all possible abnormal situations are unknown and cannot be modeled in advance. To account for this, the model builds a normal temporal model and a universal temporal model (all training sequences that may have mixture of normal and abnormal sequences). The UBM detector then compares the current phytosensor observation sequences against the normal PST model and matches the sequence against the universal PST model. If the likelihood ratio is below a threshold, then an abnormal state is detected. Note that the system can incrementally build the model during the online detection stage to make the model more adaptive to the current environment.

D. Nanoparticle Testing

For nanoparticle testing, spherical rutile TiO₂ nanoparticles (50 nm in diameter, Stock#5485MR) were purchased from Nanostructured and Amorphous Materials, Inc. (Houston, TX). The morphology and size distribution of the TiO_2 nanoparticles was conducted by the manufacturer using transmission electron microscopy and laser scattering, and provided on the certificate of analysis. Solutions of nanoparticles for toxicity testing were formed by the addition of 1 g of nanoparticles to 1 l of tap water. Tap water alone was used as a control for normal growth of the plants. The nanoparticle solution was added to the plant by pouring into the Tupperware container. As indicated earlier, each phytosensor had a separate container for the solution to prevent cross contamination. The nanoparticle and control solutions were added to the plant every two days to ensure that plants remained hydrated. This procedure was maintained throughout the course of the study.

E. Analysis of Algae Growth

After completing the initial analysis of the images, it was observed that in the control samples, there was an abundance of algae that was not present in the nanoparticle samples. Since the goal of this study was to analyze the environmental impact of the TiO₂ nanoparticles, it was important to analyze this effect. To analyze the algae growth, first the raw RGB images were segmented to separate the area containing the plants from the water only images. This segmentation left a 19×83 pixel area containing only the image of the water supply where the algae would grow, and were read into the MATLAB code. Next, the true color RGB image was converted to a grayscale intensity image by eliminating the hue and saturation information while retaining the luminance. Although the size of the image, 19 × 83 pixels was small, there were a significant number of images 891, for analysis. Due to the low resolution of the images, it was necessary to increase the size of the images by using a magnification factor of 5, increasing the image size to 95×415 pixels. This enlargement allowed smoothing of the image, which would have been impossible in the original image. After smoothing, the image was cropped with a four-element position vector [20 65 45 320], which removed the shadow effect from the corner of the water tray, and prevented any bleed over from the growing leaves in the original image. This edge cropping was necessary to reduce false positives associated with the dynamic shadows present on the edges of the container. The next step consisted of enhancing the grayscale image by transforming the values in the intensity image so that the histogram of the output image matched the specified histogram. In this study, the intensity values were originally spread over the entire range from [0, 255]. After enhancing the contrast, a threshold was set to detect the emergence of the algae; in this particular case, a value of 7 was used. All pixels with a value less than 7 were considered to be algae, and appeared as white in the resulting images. In the final step, the image stream was saved as a video, and the amount of algae over time was determined based on the number of pixels detected at each time point.

III. RESULTS

A. Effect of TiO₂ Nanoparticles on Arabidopsis Health

Experiments were conducted over 28 days, yielding 891 total frames for analysis. Using the detection system, it was possible to detect changes in the control biosensors, Biosensors 1 and 2, at day 15. In these samples, the leaves began to change from green to yellow, indicating decreasing plant health. On day 16, greater than 20% of the leaves had turned yellow, with increasing amounts of yellow observed during the continuation of the study. As indicated in Fig. 3, the change in green level in Biosensors 1 and 2 could be clearly observed. For the fuzzy ART detection, the plants were classified into two categories, either increased or decreased health. The model was tuned so that if >20% of the leaves turned yellow, the plant was deemed to be unhealthy, indicating a lack of growth. Based on the fuzzy ART detection, at 500 min Biosensors 1 and 2 changed their status from healthy to unhealthy (see Fig. 4). They remained in the unhealthy state

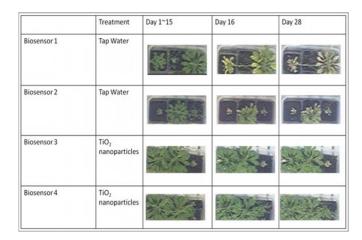


Fig. 3. Images captured during the course of the study. At Day 16, the percentage of yellow leaves was greater than 20% of the green pixels in Biosensors 1 and 2. Biosensors 2 and 4 maintained their green color throughout the course of the study.

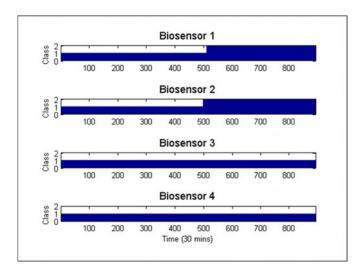


Fig. 4. Fuzzy ART neural network detection results. Class 1 indicates all plants were green. Class 2 indicates more than 20% of the leaves changed into yellow. Sampling rate is 30 min per frame. The algorithm detected changes in Biosensors 1 and 2 around day 16. Whole experiment took 28 days (891 samples).

for the remainder of the study. In contrast, Biosensors 3 and 4, the TiO_2 nanoparticle treated samples, remained in the healthy state for the duration of the study. It should be that the increase in growth of Biosensors 3 and 4 did not affect their state classification. The increased growth of the TiO_2 nanoparticle treated samples was an unexpected result, but demonstrates the strength of the sensor-network approach. The vigilance value for Biosensors 1, 3, and 4 was 0.6, while the vigilance value for Biosensor 2 was 0.77. The vigilance value is a measure of the sensitivity of the range of 0–1. 0 means that the network is not sensitive to any change, while 1 indicates that a new class would be created for each pixel change. Biosensor 2 had a higher vigilance factor because the starting plant was smaller, providing fewer green pixels. Since Biosensors 1, 3, and 4 used similar size plants, it was possible to analyze them with the same vigilance value.

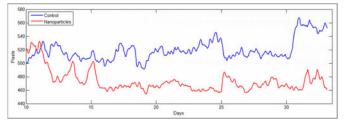


Fig. 5. Analysis of algae growth over time in both the control and nanoparticle treated samples. Note that at the completion of the study, there is significantly more algae present in the control samples. In addition, the samples begin to diverge around 12 days, coinciding with the appearance of the unhealthy state in the plant analysis. The noise introduced into the dataset is likely the result of perturbation through the addition of new aqueous solution and evaporation.

To ensure that the trends observed were reproducible, the study was replicated two more times with similar results.

B. Effect of TiO₂ Nanoparticles on Algae Growth

In addition to analysis of the terrestrial plant health, the biosensor system also allowed for visualization of algae proliferation in the solution used to maintain the hydration of the phytosensors. Analysis of the image segments that monitored the water level in the plant containers showed that there was an inverse relationship between the growth of algae in the control samples and the decreased growth of the control plants (see Fig. 5). Due to the addition of the TiO₂ nanoparticles into the experimental biosensors, there was a significant decrease in the amount of algae, indicating a toxic effect from the TiO₂ nanoparticles. Considering that the algae will compete with the terrestrial plants for the availability of nutrients, the emergence of algae in the water supply is expected to decrease the growth of the slower growing terrestrial plants. This is a common phenomenon observed with eutrophication induced from environmental contaminants, where the rapid growth of algae will outcompete slower growing plants. While the exact mechanism used by the TiO2 to reduce algae growth in the biosensors cannot be identified from this study, previous studies have demonstrated that nanoparticles are toxic to many species of green algae [48]–[50]. Clearly, the reduction in the amount of algae between the controls and experimental biosensors illustrates a potential toxic response from the introduction of the nanoparticles into the system. This environmental impact might lead to changes at other trophic levels leading to a greater environmental impact over a long time course.

IV. DISCUSSION

In this study, we have demonstrated the feasibility of using phytosensors to detect the environmental impact of nanomaterials, specifically, TiO₂ nanoparticles, in a system similar to that found in the real world. In the proof-of-concept design, the biosensor node was designed as a two component system that has a proven capability to detect the impact on both a terrestrial plant and algae. In this study, the TiO₂ nanoparticles, at a concentration of 1000 mg/ml, demonstrated an increase in the green content of these samples, potentially indicating an increase in the health of *Arabidopsis thaliana* when compared to untreated

controls. At the same time, the reverse trend was observed for the growth of green algae in the biosensor system, with the control samples having pronounced algal growth when compared to the TiO₂ treated biosensors. Several mechanisms exist in the literature to explain the observed effects from the introduction of the TiO₂ nanoparticles. Previously, TiO₂ nanoparticles have been shown to induce the isotropic growth of root cells through disregulation of microtubule assembly in Arabidopsis [51]. The increased speed of Arabidopsis growth after introduction of the TiO₂ nanoparticles may be the result of this rapid root growth, or, potentially, the response to the decreased algae content. As observed in eutrophic systems, the growth of algae can lead to a reduction in available nutrients for other slower growing plant species. It is possible that the decreased amount of algae in the experimental samples lead to more nutrient availability, and increased growth. In either instance, the goal of this study was to develop a robust environmental monitoring system that could detect any environmental impact from the introduction of the nanomaterials. In this way, the biosensor system demonstrated its strength, whereas a more specific detection system may have missed the observation of the impact.

Another key aspect of the system designed in this study is the expandability, and the ease of use of the system. The developed biosensor detection system can autonomously detect anomalies adaptively using sensor data that are collected by distributed biosensors. In the proof-of-concept design of the biosensors, the biosensor node is a two component system that has proven capable at detecting the impact on a terrestrial plant and algae. In future studies, the goal will be to expand the system to include invertebrate grazers, Daphnia, and vertebrates (fish), Danio rerio, both of which are key species used to determine aquatic toxicity [50], [52]–[54]. This will expand the platform for analysis of three trophic levels, and will also help to gauge if there is a keystone species, to which a nanoparticle effect will have a more dramatic effect. Typically, such complicated ecosystem analysis is difficult, and often requires a significant amount of time from an observer, and generates qualitative data. Because the system developed in this study uses cameras to observe changes over time, and machine learning techniques are used to automatically making sequential detection decisions, there is a decreased investment of human monitoring of the system. By monitoring the system through a sequence of image data, it is also possible to obtain quantitative data from the system, which would otherwise not be possible. As indicated earlier, it is also possible to introduce any nanomaterial into the system to determine if a potential environmental impact may result from this nanomaterial. Considering the cost effectiveness of the developed system, it would also be possible to screen a wide range of nanomaterials, and nanomaterial concentrations, in a high-throughput manner without significant monetary or labor costs. Considering the speed at which new nanomaterials are currently being created and used, a robust system for determining the environmental impact of nanomaterials is crucial.

Perhaps the most significant application for the phytosensor system developed in this study is the potential large-scale monitoring of wide geographic areas to determine the health of the system. With the advent of satellite imaging, it would be possible to survey a large area and use machine learning and image processing techniques to determine the health status of an entire crop. Using such an approach, it would be possible to determine if there is a change in crop health in near real-time and also to determine the area of the crop most affected. In the case of a toxic event, using the concept developed in this study, it would be possible to identify the toxic effect in a time-dependent manner, which would indicate where the toxin was introduced into the environment. Determination of the source of introduction of a toxin into the environment is very difficult to ascertain when on the ground, and delays in determining the source can lead to significant environmental damage, increased costs, and further spreading. Large-scale environmental monitoring, such as described earlier, would be extremely difficult without an adaptable automated system, and represents the true strength of a system such as that designed in this study.

V. CONCLUSION

Using machine learning, phytosensors, and remote monitoring techniques, we established a proof-of-concept system for monitoring the environmental impact of nanoparticles on both a terrestrial plant and algae. We observed increased leaf growth in Arabidopsis thaliana with the introduction of 1000 mg/ml TiO₂ nanoparticles, when compared to control plants. Corresponding with the occurrence of this growth trend, there was a toxic response to green algae causing a significant reduction in biomass in the TiO₂ treated samples. By using the machine learning framework, it was possible to detect this environmental impact using an automated system, without a substantial investment of time or monitoring from a human operator. The system demonstrates a cost-effective approach for the monitoring of the environmental impact of nanomaterials using sensor network and presents a scalable alternative for large-scale and wide-area monitoring. This system could be used for future studies to identify the risk associated with the large-scale use of nanomaterials.

REFERENCES

- [1] D. Berube, "Rhetorical gamesmanship in the nano debates over sunscreens and nanoparticles," *J. Nanoparticle Res.*, vol. 10, pp. 23–37, 2008.
- [2] C. Contado and A. Pagnoni, "TiO₂ in commercial sunscreen lotion: Flow field-flow fractionation and ICP-AES together for size analysis," *Anal. Chem.*, vol. 80, pp. 7594–7608, 2008.
- [3] T. Faunce, K. Murray, H. Nasu, and D. Bowman, "Sunscreen safety: The precautionary principle, the Australian therapeutic goods administration and nanoparticles in sunscreens," *NanoEthics*, vol. 2, pp. 231–240, 2008.
- [4] K. Schmid and M. Riediker, "Use of nanoparticles in swiss industry: A targeted survey," *Environ. Sci. Technol.*, vol. 42, pp. 2253–2260, 2008.
- [5] N. Sozer and J. L. Kokini, "Nanotechnology and its applications in the food sector," *Trends Biotechnol.*, vol. 27, pp. 82–89, 2009.
- [6] K. Tiede, A. Boxall, S. Tear, J. Lewis, H. David, and M. Hassellov, "Detection and characterization of engineered nanoparticles in food and the environment," *Food Additives Contaminants*, vol. 25, pp. 795–821, 2008
- [7] V. Palanivel, D. Zhu, and W. J. van Ooij, "Nanoparticle-filled silane films as chromate replacements for aluminum alloys," *Prog. Organic Coatings*, vol. 47, pp. 384–392, 2003.
- [8] E. Corredor, P. Testillano, M.-J. Coronado, P. Gonzalez-Melendi, R. Fernandez-Pacheco, C. Marquina, M. R. Ibarra, J. de la Fuente, D. Rubiales, A. Perez-de-Luque, and M.-C. Risueno, "Nanoparticle penetration"

- and transport in living pumpkin plants: In situ subcellular identification," *BMC Plant Biol.*, vol. 9, pp. 45–56, 2009.
- [9] W.-M. Lee, Y.-J. An, H. Yoon, and H.-S. Kweon, "Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (Phaseolus radiatus) and wheat (Triticum aestivum): Plant agar test for waterinsoluble nanoparticles," *Environ. Toxicol. Chem.*, vol. 27, pp. 1915–1921, 2008.
- [10] D. Lin and B. Xing, "Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth," *Environ. Pollution*, vol. 150, pp. 243–250, 2007
- [11] S. B. Lovern, J. R. Strickler, and R. Klaper, "Behavioral and physiological changes in daphnia magna when exposed to nanoparticle suspensions (Titanium Dioxide, Nano-C60, and C60HxC70 Hx)," *Environ. Sci. Technol.*, vol. 41, pp. 4465–4470, 2007.
- [12] M. Auffan, J. Rose, M. R. Wiesner, and J.-Y. Bottero, "Chemical stability of metallic nanoparticles: A parameter controlling their potential cellular toxicity in vitro," *Environ. Pollution*, vol. 157, pp. 1127–1133, 2009.
- [13] W.-S. Cho, M. Cho, S. R. Kim, M. Choi, J. Y. Lee, B. S. Han, S. N. Park, M. K. Yu, S. Jon, and J. Jeong, "Pulmonary toxicity and kinetic study of Cy5.5-conjugated superparamagnetic iron oxide nanoparticles by optical imaging," *Toxicol. Appl. Pharmacol.*, vol. 239, pp. 106–115, 2009.
- [14] S. M. Hussain, K. L. Hess, J. M. Gearhart, K. T. Geiss, and J. J. Schlager, "In vitro toxicity of nanoparticles in BRL 3 A rat liver cells," *Toxicol. Vitro*, vol. 19, pp. 975–983, 2005.
- [15] S. Kim, J. E. Choi, J. Choi, K.-H. Chung, K. Park, J. Yi, and D.-Y. Ryu, "Oxidative stress-dependent toxicity of silver nanoparticles in human hepatoma cells," *Toxicol. Vitro*, vol. 23, pp. 1076–1084, 2009.
- [16] W. Lin, Y.-w. Huang, X.-D. Zhou, and Y. Ma, "In vitro toxicity of silica nanoparticles in human lung cancer cells," *Toxicol. Appl. Pharmacol.*, vol. 217, pp. 252–259, 2006.
- [17] C. Migdal, R. Rahal, A. Rubod, S. Callejon, E. Colomb, N. Atrux-Tallau, M. Haftek, C. Vincent, M. Serres, and S. Daniele, "Internalisation of hybrid titanium dioxide/para-amino benzoic acid nanoparticles in human dendritic cells did not induce toxicity and changes in their functions," *Toxicol. Lett.*, vol. 199, pp. 34–42, 2010.
- [18] M. V. D. Z. Park, W. Annema, A. Salvati, A. Lesniak, A. Elsaesser, C. Barnes, G. McKerr, C. V. Howard, I. Lynch, K. A. Dawson, A. H. Piersma, and W. H. de Jong, "In vitro developmental toxicity test detects inhibition of stem cell differentiation by silica nanoparticles," *Toxicol. Appl. Pharmacol.*, vol. 240, pp. 108–116, 2009.
- [19] J. Wu, W. Liu, C. Xue, S. Zhou, F. Lan, L. Bi, H. Xu, X. Yang, and F.-D. Zeng, "Toxicity and penetration of TiO2 nanoparticles in hairless mice and porcine skin after subchronic dermal exposure," *Toxicol. Lett.*, vol. 191, pp. 1–8, 2009.
- [20] L. Xia, S. Lenaghan, M. Zhang, Z. Zhang, and Q. Li, "Naturally occurring nanoparticles from english ivy: An alternative to metal-based nanoparticles for UV protection," *J. Nanobiotechnol.*, vol. 8, pp. 1–9, 2010.
- [21] U. M. Dhana lekshmi, G. Poovi, N. Kishore, and P. N. Reddy, "In vitro characterization and in vivo toxicity study of repaglinide loaded poly (methyl methacrylate) nanoparticles," *Int. J. Pharmaceutics*, vol. 396, pp. 194–203, 2010.
- [22] G. Huang, N. Zhang, X. Bi, and M. Dou, "Solid lipid nanoparticles of temozolomide: Potential reduction of cardial and nephric toxicity," *Int. J. Pharmaceutics*, vol. 355, pp. 314–320, 2008.
- [23] R. Kedmi, N. Ben-Arie, and D. Peer, "The systemic toxicity of positively charged lipid nanoparticles and the role of Toll-like receptor 4 in immune activation," *Biomaterials*, vol. 31, pp. 6867–6875, 2010.
- [24] J.-P. Plard and D. Bazile, "Comparison of the safety profiles of PLA50 and Me.PEG-PLA50 nanoparticles after single dose intravenous administration to rat," *Colloids Surfaces B: Biointerfaces*, vol. 16, pp. 173–183, 1999.
- [25] B. Semete, L. Booysen, Y. Lemmer, L. Kalombo, L. Katata, J. Verschoor, and H. S. Swai, "In vivo evaluation of the biodistribution and safety of PLGA nanoparticles as drug delivery systems," *Nanomedicine: Nanotechnol., Biol. Med.*, vol. 6, pp. 662–671, 2010.
- [26] P. Singh, D. Prasuhn, R. M. Yeh, G. Destito, C. S. Rae, K. Osborn, M. G. Finn, and M. Manchester, "Bio-distribution, toxicity and pathology of cowpea mosaic virus nanoparticles in vivo," *J. Controlled Release*, vol. 120, pp. 41–50, 2007.
- [27] D. B. Kane, B. Oktem, and M. V. Johnston, "Nanoparticle detection by aerosol mass spectrometry," *Aerosol Sci. Technol.*, vol. 34, pp. 520–527, 2001
- [28] B. Simonet and M. Valcárcel, "Monitoring nanoparticles in the environment," *Anal. Bioanal. Chem.*, vol. 393, pp. 17–21, 2009.

- [29] R. P. Hollis, K. Killham, and L. A. Glover, "Design and application of a biosensor for monitoring toxicity of compounds to eukaryotes," *Appl. Environ. Microbiol.*, vol. 66, pp. 1676–1679, Apr. 1, 2000.
- [30] I. Bontidean, A. Mortari, S. Leth, N. L. Brown, U. Karlson, M. M. Larsen, J. Vangronsveld, P. Corbisier, and E. Csöregi, "Biosensors for detection of mercury in contaminated soils," *Environ. Pollution*, vol. 131, pp. 255–262, 2004
- [31] M. T. Giardi, M. Koblízek, and J. Masojídek, "Photosystem II-based biosensors for the detection of pollutants," *Biosens. Bioelectron.*, vol. 16, pp. 1027–1033, 2001.
- [32] U. Bilitewski and A. P. F. Turner, Eds., Biosensors for Environmental Monitoring. New York: Taylor & Francis, 2000.
- [33] B. Sanchez, G. Yale, R. Chatni, H. Ochoa-Acuña, D. Porterfield, E. Mclamore, and M. Sepúlveda, "Oxygen flux as an indicator of physiological stress in aquatic organisms: A real-time biomonitoring system of water quality," *Proc. SPIE*, vol. 7304, pp. 730405-1–730405-8, 2009.
- [34] A. Milenkovi, C. Otto, and E. Jovanov, "Wireless sensor networks for personal health monitoring: Issues and an implementation," *Comput. Commun.*, vol. 29, pp. 2521–2533, 2006.
- [35] L. Perfus-Barbeoch, N. Leonhardt, A. Vavasseur, and C. Forestier, "Heavy metal toxicity: Cadmium permeates through calcium channels and disturbs the plant water status," *Plant J.*, vol. 32, pp. 539–548, 2002.
- [36] N. Terry, "Physiology of trace element toxicity and its relation to iron stress," J. Plant Nutrition, vol. 3, pp. 561–578, 1981.
- [37] C. Lee, S. Mahendra, K. Zodrow, D. Li, Y. Tsai, J. Braam, and P. Alvarez, "Developmental phytotoxicity of metal oxide nanoparticles to arabidopsis thaliana," *Environ. Toxicol. Chem.*, vol. 29, pp. 669–675, 2010.
- [38] C.-X. Shen, Q.-F. Zhang, J. Li, F.-C. Bi, and N. Yao, "Induction of programmed cell death in arabidopsis and rice by single-wall carbon nanotubes," *Amer. J. Botany*, vol. 97, pp. 1602–1609, Oct. 1, 2010.
- [39] L. Chaerle, I. Leinonen, H. G. Jones, and D. Van Der Straeten, "Monitoring and screening plant populations with combined thermal and chlorophyll fluorescence imaging," *J. Exp. Botany*, vol. 58, pp. 773–784, Mar. 1, 2007.
- [40] Y. Li, , M. Thomason, and L. E. Parker, "Detecting time-related changes in wireless sensor networks using symbol compression and probabilistic suffix trees," in *Proc. IEEE/RSJ Int. Conf. Int. Robots Syst.*, 2010, pp. 2946–2951.
- [41] A. Kulakov and D. Davcev, "Tracking of unusual events in wireless sensor networks based on artificial neural-networks algorithms," in *Proc. Int. Conf. Inf. Technol.: Coding Comput.*, 2005, vol. 02, pp. 534–539.
- [42] Y. Li and L. E. Parker, "Detecting and monitoring time-related abnormal events using a wireless sensor network and mobile robot," in *Proc. IEEE/RSJ Int. Conf. Int. Robots Syst.*, 2008, pp. 3292–3298.
- [43] Y. Li and L. E. Parker, "A spatial-temporal imputation technique for classification with missing data in a wireless sensor network," in *Proc. IEEE/RSJ Int. Conf. Int. Robots Syst.*, 2008, pp. 3272–3279.
- [44] K. Sayood, Introduction to Data Compression. San Francisco, CA, USA: Morgan Kaufmann, 2000.
- [45] G. Mazeroff, J. Gregor, M. Thomason, and R. Ford, "Probabilistic suffix models for API sequence analysis of windows XP applications," *Pattern Recog.*, vol. 41, pp. 90–101, 2008.
- [46] D. Ron, Y. Singer, and N. Tishby, "The power of amnesia: Learning probabilistic automata with variable memory length," *Mach. Learn.*, vol. 25, pp. 117–149, 1996.
- [47] D. A. Reynolds, T. F. Quatieri, and R. B. Dunn, "Speaker verification using adapted Gaussian mixture models," *Digital Signal Process.*, vol. 10, pp. 19–41, 2000.
- [48] V. Aruoja, H.-C. Dubourguier, K. Kasemets, and A. Kahru, "Toxicity of nanoparticles of CuO, ZnO and TiO₂ to microalgae pseudokirchneriella subcapitata," *Sci. Total Environ.*, vol. 407, pp. 1461–1468, 2009.
- [49] K. Hund-Rinke and M. Simon, "Ecotoxic effect of photocatalytic active nanoparticles (TiO₂) on algae and daphnids (8 pp)," *Environ. Sci. Pollution Res.*, vol. 13, pp. 225–232, 2006.
- [50] S. Hall, T. Bradley, J. T. Moore, T. Kuykindall, and L. Minella, "Acute and chronic toxicity of nano-scale TiO₂ particles to freshwater fish, cladocerans, and green algae, and effects of organic and inorganic substrate on TiO₂ toxicity," *Nanotoxicology*, vol. 3, pp. 91–97, 2009.
- [51] S. Wang, J. Kurepa, and J. A. Smalle, "Ultra-small TiO₂ nanoparticles disrupt microtubular networks in arabidopsis thaliana," *Plant, Cell Environ.*, vol. 34, pp. 811–820, 2011.
- [52] X. Zhu, Y. Chang, and Y. Chen, "Toxicity and bioaccumulation of TiO₂ nanoparticle aggregates in daphnia magna," *Chemosphere*, vol. 78, pp. 209–215, 2010.

- [53] X. Zhu, L. Zhu, Z. Duan, R. Qi, Y. Li, and Y. Lang, "Comparative toxicity of several metal oxide nanoparticle aqueous suspensions to zebrafish (Danio rerio) early developmental stage," *J. Environ. Sci. Health, Part A*, vol. 43, pp. 278–284, 2008.
- [54] R. J. Griffitt, J. Luo, J. Gao, J.-C. Bonzongo, and D. S. Barber, "Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms," *Environ. Toxicol. Chem.*, vol. 27, pp. 1972–1978, 2008



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