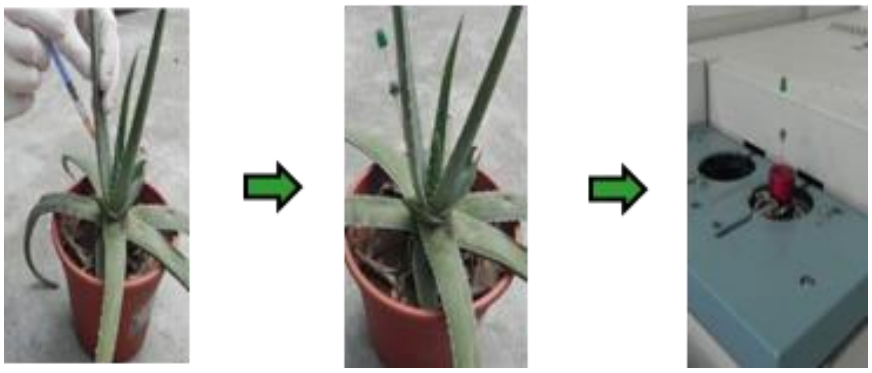
In Vivo Tracing of The Effect of Microplastic Pollution on Salicylic Acids and Organophosphorus Pesticides Uptake in Aloe



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Abstract

As a new type of environmental pollutant, microplastics has received worldwide attention in recent years and has been listed as the second largest scientific issue in the field of environmental and ecological science research. Plants closely interact with environmental elements such as soil, water and atmosphere, and they are the starting point for the bioaccumulation of toxic substances. To study the effect of microplastics on the plants growth, target analytes of trace amounts have to be isolated and enriched before analysis. However, at present, the sampling treatment often needs to destroy the plants, and the growth process of plants cannot be traced. This is the major obstacle that retards the study of the effects of microplastics on physiological activities of plants.

In view of the above problems, this study adopts a nondestructive sampling technique of solid phase microextraction (SPME) to in vivo sample the living plants, and to trace the concentration change of the target analytes of endogenous salicylic acid (SAs) and organophosphorus pesticides(OPPs). Two kinds of efficient SPME coating were designed and prepared to meet the requirement of different analytes. Aloe vera with stem and leaf hypertrophy was selected as a representative of plants. Two

kinds of micro-plastics which were nano-polystyrene (PS) existed in artificial soil and acrylic polymer spheres (PBA) existed in wastewater were investigated on their effects on SAs and OPPS uptake in aloe.

The results show that in vivo SPME method featured low LODs and LOQs, wide linear ranges, as well as high accuracy and repeatability. The in vivo tracing of the targeted analytes in aloe leaves under microplastic stress showed that SAs, plant's regulator hormones, upsurged to activate the defense system against abiotic stress of microplastics. In addition, microplastics can increase the rate of OPPs access plants and more OPPs uptake in aloe under microplastics stress. Due to the nonlethal and simple operation, the result of the experiment identified microplastics as a source of bio-stress and discovered certain characteristics of microplastics as a pollutant.

Keyword: Microplastic pollution; Aloe; in vivo SPME ; Salicylic acid (SAs) ; Organophosphorus pesticides(OPPs).

Statement of Originality

The research process and result of this team are conducted and derived under the guidance of the instructor. Other than the referenced content and the acknowledged sources, this paper does not include any published findings by this group or any other researchers. If there is any inaccuracy, this team is accountable for all liabilities.

Signature:

Date:

1. Introduction

1.1 Current Situation of Microplastics Pollution

Microplastics, as an emerging environmental pollutant, pose a serious threat to the global ecosystem. According to the National Oceanic and Atmospheric Administration (NOAA), microplastics is plastic particles less than 5mm in size.[1] It is estimated that about 150 million tons of waste plastics "disappear" each year as they are weathered or brittle into fine particles under natural forces such as water flow and wind.[2] The United Nations estimates that there are 46,000 microplastics per square mile of ocean, and there are a total of 51 trillion microplastic particles in the world's oceans and soil (five times the number of stars detected across our entire galaxy).[3] In 2015, microplastics pollution was listed as the second largest scientific issue in the field of environmental and ecological science research and became a major global environmental issue alongside global climate change, ozone depletion and ocean acidification.

Figure.1 The microplastic “monster was released from the bottle”

Plastics are difficult to degrade. Even when plastics are broken down into small particles, they still exist in the form of microplastics or nanoplastics accumulating in the ecosystem. The damage of microplastics lies in its small size, quantity, wide distribution, and certain adsorption characteristics. Microplastics can adsorb contaminants or microorganisms on its surfaces, creating a new type of pollutant that can be easily digested by organisms. Nanoplastics with smaller size can even be absorbed into biological tissue cells.[4] As microplastics accumulate in the food chain, it poses harms to the organisms at the top of the food chain and human.

Current researches on microplastic pollution mainly focus on the aquatic ecosystem such as ocean, coastal tidal beach, estuary and lake. The reason is because microplastics are easily eaten by aquatic animals. There have been reports of deaths from ingesting microplastics, and microplastic residues have been found in the guts of marine organism.[5-6] Scientists first photographed plankton ingest microplastics in 2017, confirming that discarded microplastics not just hurt larger animals, but have spread to the bottom of the food chain. A "clean ocean" campaign has been launched by United Nations Environment Programme (UNEP).[7]

But microplastics in soil may be a more serious problem. A Norwegian study estimates that 110,000 to 730,000 tons of microplastics

enter agricultural soil each year in Europe and North America.[8] But we know almost nothing about microplastics in soil, and the impact of microplastic pollution on plants remains a matter of debate and speculation. Relating studies are rare, mainly because it is commonly recognized that plants have a natural mechanism for isolating foreign foreign objects from their sporocarp.[9] Therefore, it is generally believed that microplastics cannot pass through plant cell membranes. Relevant departments have even classified microplastics as harmless garbage for agricultural use.

1.2 Main Sources of Microplastics and How Does It Get Into Agricultural Soil

There are three main source of microplastics.[10] First, microplastics was produced when plastic wastes that were left in the environment been weathered, embrittled and broken down into smaller particles under natural forces such as water flow, wind and soil microorganisms. Second, microplastics comes from plastic microspheres added to cosmetics and personal cleaning products. Third, microplastics comes from industrial or private waste water, such as waste water from washing machine, which contains the fine fiber particles that broken away from fiber clothes.

So how does microplastics enter agricultural soil? The main sources are waste-water irrigation, sludge and plastic mulch. Domestic sewage, industrial waste water and rainwater collected by sewage treatment plants

all contain microplastics. Also, during sewage treatment, the larger microplastics are filtered from the water and left in the sludge, which is then applied and dispersed on agricultural land. What's more, microplastics from fragments of agricultural plastic films can be broken down into small pieces such as nanoplastics.[11]

Potential sources of microplastics in agricultural soils also include industrial composting. National standards for compost accept microplastics to be visible contamination" in products. As the greening of walls and roofs has been widely adopted for more architectures, polypropylene (PS) microparticles have been specifically used to make lightweight "soil" which is applied to farmland after been abandoned.[12]

At present, the research on soil microplastics is still in its infancy, mainly focusing on the isolation and detection of microplastics in soil and the impact of microplastic pollution on geobiont.[10] Few studies have been conducted to study the effect of microplastics on plant growth systematically.

1.3 In Vivo Solid-phase Microextraction (SPME) technique

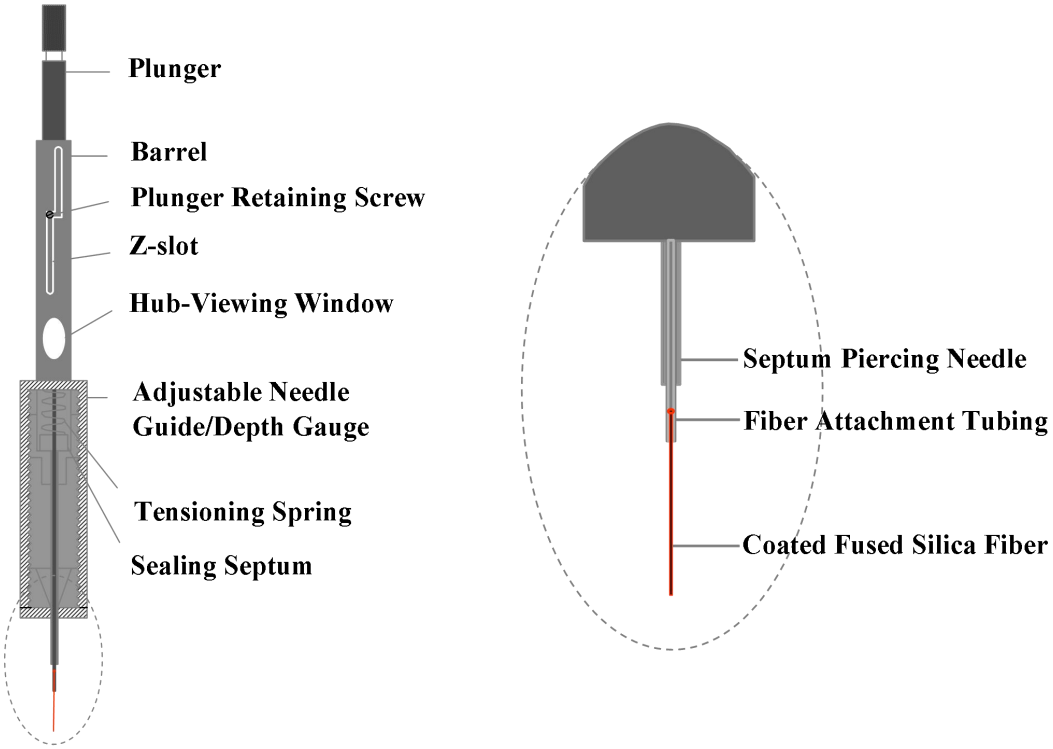


Figure.2 Typical structure of Solid-phase Microextraction fibers[14]

Solid-phase microextraction, (SPME) was first introduced by Professor Pawliszyn of Waterloo University in 1990.[13] The fused quartz fiber coated with different compounds was used as the extractor to adsorb trace organic compounds in the sample (Figure 2)[14]. Based on the distribution process of the analyte between the stationary phase of the coating and the sample matrix, the separation and gathering of the target analyte can be achieved. Then the adsorbate substance was desorbed for chromatographic analysis. SPME is a method integrating of sampling, extraction, concentration and sample injection and requires no organic solvent. With high sensitivity and easy operation, it can be directly used for the analysis of gas chromatography (GC), liquid chromatography (LC) and atomic absorption spectrometry (AAS).

The extraction coating material is the most important factor affecting the extraction efficiency of SPME, and the analyte acts on the stationary phase according to the principle of "similar compatibility".[15] Different coating material results in different range of adaptation to extract-enriched analytes. Therefore, the choice of SPME coating material should be based on the physicochemical properties of the analyte (e.g partition coefficient, volatility and polarity). Generally, for non-polar or weakly polar compounds, polydimethylsiloxane (PDMS) coatings, such as hydrocarbons and hydrocarbon derivatives, are commonly chosed. For polar compounds containing heteroatoms, polyacrylic acid etc. Ester coating (PA) is often needed. For more complex analytical systems, some composite coatings (such as PDMS/DVB, CAR/PDMS) can be used.[16]

According to the requirements of target analytes, extraction conditions and chromatographic operating conditions should be optimized[17] after suitable extraction fiber was gotten.

1.4 The Purpose of This Research AND Research Planning

Based on the above research overview, it is found that few studies are been conducted on the effects of microplastic pollution on soil ecosystems. Up to now, systematic studies on the effects of microplastics on plant growth has not been reported. However, due to the landfill, industrial production, human life and the development of agricultural technology, microplastics enter the terrestrial ecosystem in large

quantities. Due to their adsorption characteristics, the microplastics can not only adsorb organic pollutants, but also serve as heavy metal carriers. In addition, microplastics can also change the physical properties of the soil and accumulate to a certain concentration in the soil, which will have an impact on soil function and biodiversity. In particular, a large number of 1-100nm microplastics have been found in the soil in recent years, and it has been found that nanoplastics can be absorbed by the roots of plants.[18] Plants are closely related to environmental factors such as soil, water and atmosphere. They are the starting point for the bioaccumulation of toxic substances. Therefore, it is urgent to systematically study the effects of microplastics on plant growth in soil.

Phytohormone are important endogenous-active substances that regulate growth development and differentiation of plants.[19] Once the micro-plastics in the soil affect plant growth, it will disturb the normal physiological activity of the plants. Therefore, studying the regulation mechanism of microplastics on endogenous active substances such as plant hormones in soil is an important direction to study the influence of soil micro-plastics on plant physiological activities at the molecular level. Salicylic acid (SA) and derivatives are a kind of stressed plant regulators, [20] which can sensitively reflect the influence of microplastic pollution on plant growth and development. Therefore, salicylic acid (SA) and acetylsalicylic acid (ASA) can be selected as representative endogenous

analytes that would act as an indicator of the bio-stress posed by microplastic pollution on the plants.

However, SAs has negative charge and is difficult to extract.[21] At present, the research mainly uses destructive sampling method which is difficult to systematically track and monitor the influence of microplastics.[22-24] Nondestructive sampling technique of in vivo SPME can be applied to track the concentration variation of analytes during monitor process, more accurately reflecting the dynamic response of endogenous active substances in plants under stress of microplastic pollution.

Studying the effects of soil microplastics on the uptake of organic pollutants in plant is another important aim due to micro-plastics, especially nanoplastics, having surface effects can adsorb and enrich organic pollution. Organophosphorus pesticides (OPPs) are the most widely used pesticides in agriculture, most of which are moderately toxic or highly toxic.[25] Due to the excessive use in agriculture, OPPs have become a new type of organic pollutants in recent years. OPPs that remain in the soil or water by spraying and the like are easily transmitted into the human body through the food chain, which seriously endangers human health.[26] Therefore, OPPs can be used as representative target analytes to study the effects of microplastics on the uptake of organic pollutants in plants by in vivo SPME.

To this end, this research project plans to nondestructive sample of living plants by in vivo SPME to trace the impact of soil microplastic pollution on plant through monitoring concentration change of plant regulators and organopollution residues.

The research content includes:

(1) Salicylic acid (SA) and acetylsalicylic acid (ASA), as well as three organophosphorus pesticides(OPPs) of propetamphos, parathion-methyl and quinalphos were selected as target analytes. Two kinds of efficient SPME coating were designed and prepared for different target analytes. The structure of the coating material are characterized.

(2) The coating materials were coated on different substrates (quartz fiber and steel wire, respectively) to prepare two SPME fiber probes, and the morphology, reproducibility and detection limit of the probes were studied.

(3) Aloe vera with hypertrophic leaves was selected as a representative of plants. According to the investigation on the source of microplastics in the local soil, two kinds of representative microplastics of nano-polystyrene (PS existed in sludge and artificial soil) and acrylic Carbomer microspheres (PBA existed in cosmetics and detergents) were selected. Exposure experiments are conducted. Sampling is carried out daily, and after the analyte concentration reached equilibrium, aloes were removed from microplastic environment and the elimination experiment

was performed.

(4) After in vivo sampling using SPME, liquid chromatography with photodiode array detector (LC-PAD) is used to analyses non-volatile SAs, and gas chromatography-mass spectrometry (GC-MS) is applied to volatile and semi-volatile OPPs. The effects of microplastics pollution on the uptake and elimination of endogenous phytohormones and organophosphates in aloe were evaluated.

(5) A "Clean Soil" proposal about research on enzyme degradation of microplastics was proposed.

In this research, a in vivo SPME bio-sampling technique is put forward to trace and monitor the dynamic change of uptake, accumulation, elimination and residue behavior of endogenous phytohormones and organpollution in plants under the influence of micro-plastic pollution. The mechanism of the influence of microplastics pollution on plant growth is explored. Carrying out this research has important theoretical and practical significance for comprehensive evaluation of the biological effects of soil microplastic pollution, for assessment of environmental risks and human health risks of microplastics.

2. Preparation of In Vivo Solid-Phase Microextraction(SPME) Fibers

2.1 Experimental Materials and Instruments

2.1.1 Chemicals and Materials

Dimethylformamide (DMF), N,N-dicyclohexylcarbodie (DCC) and diallyl dimethyl ammonium chloride (PDDA) were purchased from Aladdin Reagent (Shanghai, China). Polyacrylonitrile (PANI), norepinephrine (NE) and graphene oxide (GO) were purchased from J&K scientific Ltd. (Beijing, China). HPLC-grade methanol, tri (hydroxymethyl)aminomethane hydrochloride (Tris buffer) and 3.5μm-C18 particles were purchased from Sigma-Aldrich Co. Ltd. (St. Louis, USA). The quartz fiber (660 μm O.D.) was obtained from Scitlion Technology Co.Ltd. (Beijing, China). Two kinds of polydimethylsiloxane (PDMS) tubings (i.d. 0.212 mm, o.d 0.40 mm and i.d. 0.31 mm, o.d. 0.64 mm) were purchased from Helixmark (Carpinteria, CA, USA), and stainless steel wires (127 and 480 μm in diameter, medical grade) were purchased from Small Parts (Miami Lakes, FL, USA). Other common reagents such as ethanol etc. were obtained from Guangzhou Reagent Company (Guangzhou, China).

2.1.2 Instruments

(a) (b)

Figure 3 Fourier infrared spectrometer (a) and scanning electron microscopy (b) used in the studies

Fourier infrared spectrometer (FTIR, Tensor 27, Bruker,Germany) in attenuation total reflection (ATR) mode was used to characterize the structure of Coating (Figure 3a). Scanning electron microscopy (SEM) images of the samples were obtained using a Hitachi S-4800 field emission electron microscope equipped with an energy dispersive X-ray fluorescence spectrometer for element analysis (Figure 3b).

2.2 Design Principle of SPME Fibers

The performance of SPME fiber is critically dependent on the extraction phase, namely, the fiber coatings. Commonly used PDMS coating fibers suitable for detecting volatile and semi-volatile analytes can be used for in vivo sampling of OPPs uptake in Aloe, however preparation of SPME fibers for sampling highly polar compounds of SAs in living plants is quite challenging. The coatings for sampling endophytic compounds should be biocompatible and resistant to matrix effects to avoid rejection reactions and binding of biomacromolecules [27]. Therefore, a novel SPME coating combined with the absorptive properties toward highly polar compounds and excellent biocompatibility against matrix effects is in great demand.

Inspired by previous studies on in vivo monitoring of acidic

pharmaceuticals in fish [28], a SPME coating was prepared by poly (diallyldimethylammonium chloride) (PDDA) assembled graphene oxide (GO)-coated C18 (C18@GO@PDDA) composite particles. C18 is a type of surface modified amorphous silica particles that has good adsorption ability and is widely used in solidphase extraction and solid-phase microextraction. GO has a polar and hydrophilic character. PDDA is a cationic polyelectrolyte agent with a strong positive charge, which leads to the generation of strong electrostatic attractions between the adsorbent and acidic compounds. We anticipated that the specific properties of GO and PDDA would better enhance the absorptive ability of C18. Then the C18@GO@PDDA coating was glued onto a quartz fiber with polyaniline (PANI). The fiber surface coating was sequentially modified with bioinspired polynorepinephrine, which provided a smooth biointerface and makes the coating suitable for in vivo sampling for aleo. The schematic drawing for structure and preparation of C18@GO@PDDA

fibers is shown in Figure 4.

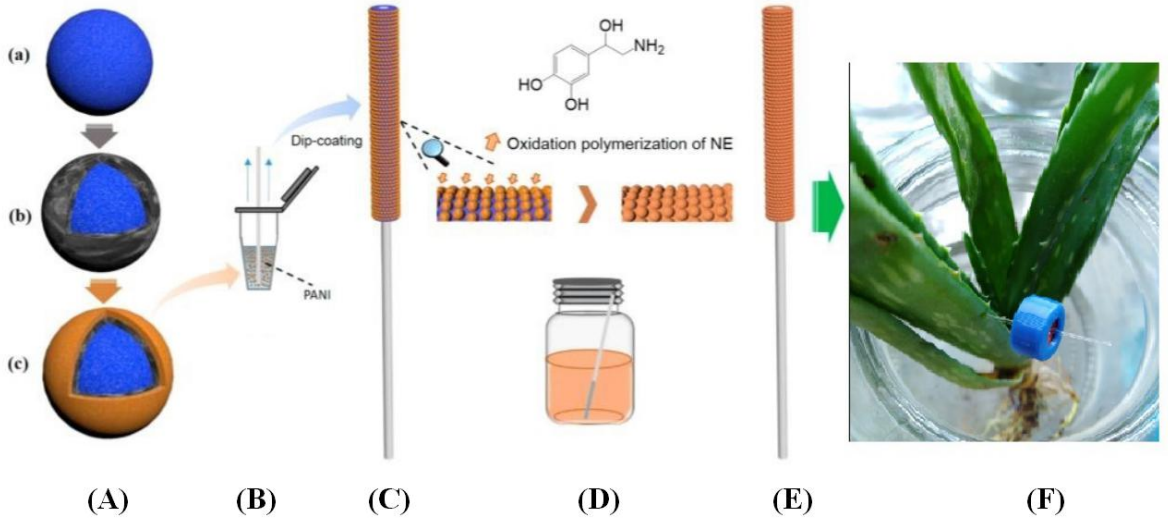


Figure 4 The schematic drawing for structure and preparation of C18@GO@PDDA fibers

2.3 Preparation of the Custom-Made SPME Fibers for In Vivo Tracing of SAs in Aloe

2.3.1 Preparation of the C18@GO@PDDA composites.

The preparation process of C18@GO@PDDA composites was preliminarily designed according to a previous study[28] and then optimized on the basis of the feasibility and the morphology of the composites. The optimized process can be briefly described as follows: 10mg of GO was added to 10mL of DMF, which was ultrasonicated for 2h. When the GO was well-dispersed, 20mg of C18 particles and 10mg of DCC were added. Then, the mixture was stirred at 500rpm at 40 °C for 24h to bond the GO to the C18 particles. Then, 10mg of C18@GO particles was added into 10g of a 2 wt % PDDA solution and dispersed by ultrasonication. The suspension was continuously stirred for 5h at 60°C to

form C18@GO@PDDA (Figure 4A).

2.3.2 Preparation of the Custom-Made Fibers.

Quartz fibers were cut into 4−5 cm segments followed by sonication in water, methanol, and acetone. After sonication, the fibers were then soaked in 0.1 M sodium hydroxide for 30 min to activate the surface, and the excess sodium hydroxide was then neutralized with hydrochloric acid. Finally, the fibers were dried at room temperature. The procedure of fabricating the custom-made fibers was as follows [29]: First, 100mg PAN was added into 1g anhydrous DMF in a 1.5mL plastic tube. The suspension was ultrasonicated until PAN particles were fully dissolved. Second, 20mg C18@GO@PDDA composite was added to the dissolved solution, and the mixture was firmly stirred and sonicated for 30min to form a dispersed slurry (Figure 4B). Third, the pretreated quartz fibers were vertically dipped into the slurry, then withdrawn slowly so that a uniform coating was covered on the surface of fibers. At last, the fibers were transferred to the drying oven with flowing nitrogen and cured for 1h at 80°C, which helped evaporate DMF and ensured better adherence of the coating on the quartz fibers (Figure 4C).

The PNE modification was processed according to the following procedures[28]: First, NE was dissolved in a mixed solvent of tris buffer (10mM, pH=8.5) (Figure 4D); then, the coated fibers were immersed in the NE solution ($0.2 gmL^{-1}$) for 16h, to form an even biocompatible

surface on the coating(Figure 4E).

2.4 Preparation of the Custom-Made SPME Fibers for In Vivo Tracing of OPPs Uptake in Aloe

Although there is commercial PDMS Fibers for SPME, the size of the in vivo fibers should be small to reduce the invasiveness imparted on the living aloe and make sure the fibers don't break during sampling at the same time. A custom-made SPME fibers of PDMS coated onto stainless steel wire were made in the laboratory as follows: [30] a piece of stainless steel wire with a length of 3 cm was cut and sonicated in acetone and deionized water for 15 min successively to remove the impurity, then the stainless steel wire was dried at room temperature and coated with a thin layer of epoxy glue for about 1 cm at one end. A piece of well-cut PDMS tubing (1.0 cm) was worn on the stainless steel wire and dried in the air at room temperature for 24h until the glue was solidified completely. The prepared PDMS fibers were conditioned in nitrogen flow at 250 °C for 15 min prior to use.

2.5 Characterizing of Custom-made SPME Fibers

2.5.1 Structure of C18@GO@PDDA coating

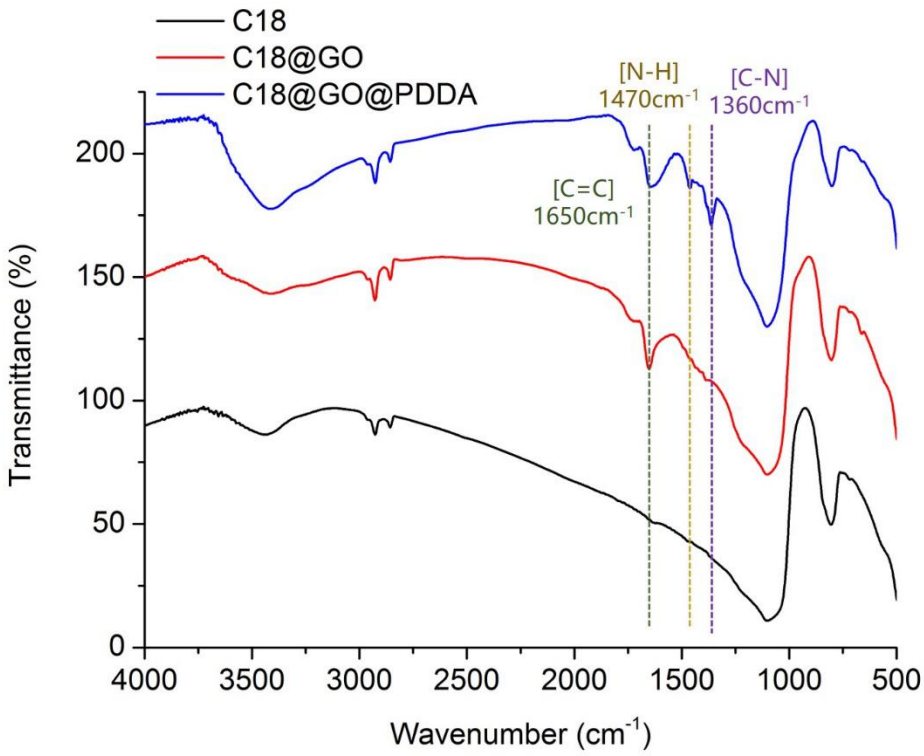


Figure 5 FTIR Spectra of C18@GO@PDDA coating

For C18@GO@PDDA coating, FTIR spectra helped confirm the presence of GO and PDDA on C18 particles (Figure 5). The three different curves denoted the IR spectra of C18 particles (black), GO-coated C18 composite (red) and PDDA-assembled GO-coated C18 composite (blue), respectively. Compared with the black curve, the absorbance at 1650cm^{-1} appeared on the other two curves iscorresponding to C＝C stretching vibrations of GO. This meant that GO was bonded onto C18 particles. Two more adsorption peaks were observed from the blue curve: The peaks at 1470 and 1360cm−1 corresponding to the N-H bending vibrations and the C-N stretching vibrations, which confirmed the bonding of PDDA. In this way, it was confirmed that C18@GO@PDDA was successfully synthesized.

2.5.2 The appearance and morphology of C18@GO@PDDA SPME Fibers

The appearance and morphology of C18@GO@PDDA SPME Fibers were observed first.

Figure 6 The appearance and assembly of C18@GO@PDDA SPME Fibers

The custom-made fibers of C18@GO@PDDA were black in appearance, because of the properties of GO, one of the main components of the fiber coating (Figure 6).

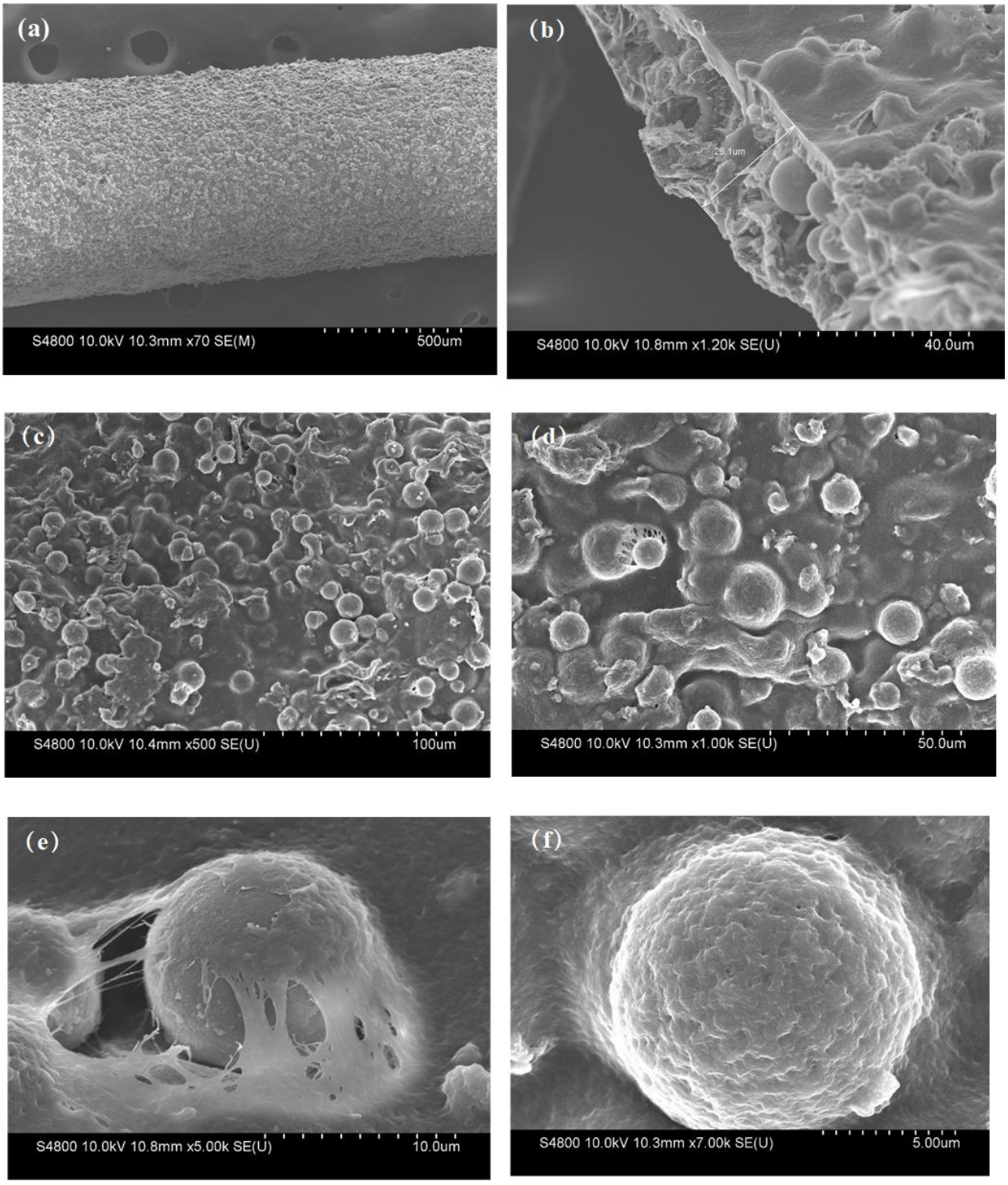
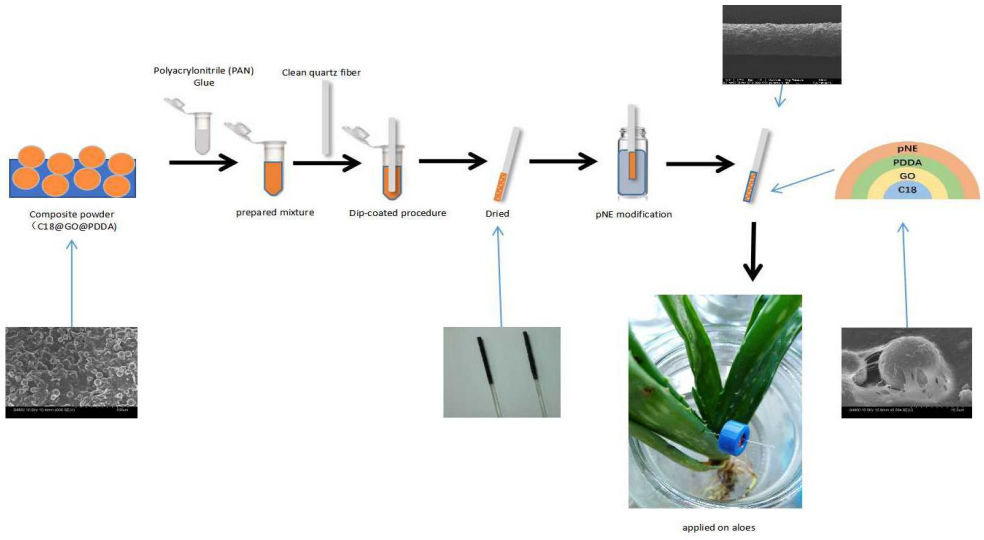


Figure 7 SEM photos of C18@GO@PDDA SPME Fibers

The appearance and morphology of C18@GO@PDDA SPME Fibers were observed. The custom-made fibers of C18@GO@PDDA were black in appearance, because of the properties of GO, one of the main components of the fiber coating (Figure 6). With the aid of SEM, we could observe the morphologies of the custom-made fiber coating under different magnification factors (Figure 7). The surface of fibers looks rough and dense under low magnification (Figure 7a) and average thickness of the fiber coating was approximately 25-30μm (Figure 7b). As magnified the observed area, we could notice that there were sphere particles dispersed evenly in the coated fiber (Figure 7c, d). It is interesting to see that sphere particles has multilayered and porous structures(Figure 7e, f), which attributed to the sol-gel coating technology[31]. It is confirmed that the anticipated structure and morphology of C18@GO@PDDA SPME fiber probes has been acquired. Stucture and morphology evolution of C18@GO@PDDA SPME fibers is



shown in Figure 8. Structure-controlled probes are a prerequisite for

successful in vivo monitoring of SAs in living plants under expose to microplastics

Figure 8 Schematic drawing of structure and morphology evolution of C18@GO@PDDA fibers

3. In Vivo Tracing of The Effect of Microplastics on SAs in Aloe

3.1 Experimental Materials and Instruments

3.1.1 Chemicals and Materials

HPLC-grade methanol was purchased from Sigma-Aldrich Co. Ltd. (St. Louis, USA). Nanospheres of Polystyrene (nano-PS C122131) with size of 100nm was purchased from Aladdin Reagent (Shanghai, China). Microspheres of acrylic resin (PBA, Carbomer 941) with size of 1-10μm and apparent density of 0.21g/cm^3 was supplied by Tinci Materials Technology Co., Ltd.(Guangzhou, China).

3.1.2 Analytical Instrument

The Breeze™ HPLC System (Waters, Singapore) consisted of a 1525 Binary HPLC Pump and a 2998 photodiode array detector (LC-PAD). A Zorbax Eclipse XDB-C18 column (4.6mm×250mm, 5μm, Agilent Technologies, CA, USA) was used for separation. The mobile phase was methanol-PBS buffer (pH=8, 70:30, v/v) with the flow rate of 0.9mL min^{-1} and the detection temperature of 25°C. The detection wave-length was 226nm. Spectra were acquired in the range of

Figure 9 The Breeze™ HPLC System

3.2 Aloe exposure experiment

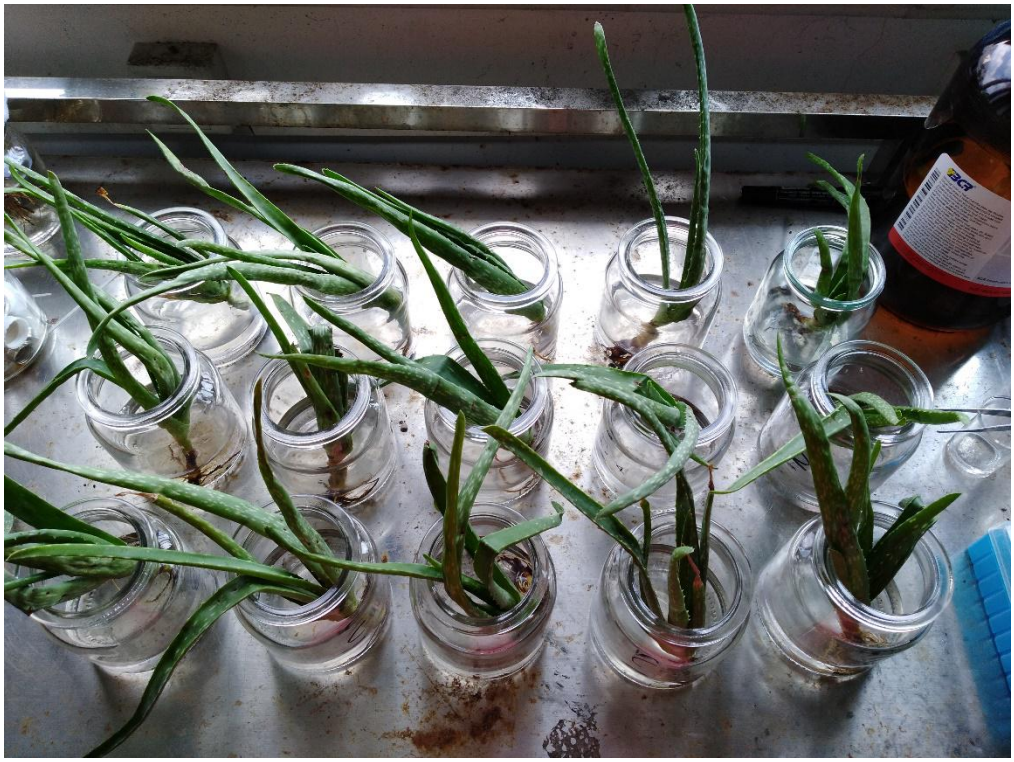


Figure 10 Aloe exposure experiment

All the aloe plants used were purchased from a local nursery. To inspect the effect of microplastics on endogenic salicylic acids, a strategy was taken by cultivated aloe plants in purified water to exclude influence of other factors. The water quality (pH, dissolved oxygen, and temperature) was monitored daily. For the exposure experiment, two experimental groups(each containing five aloes) are cultivated in 200ml

of 20ppm of microplastics added to two experimental groups, and one control group (also containing five aloes) are cultivated in 200ml of clear water.

3.3 Method optimization

Method optimization was carried out by using spiked homogenized tissues of the aloe leaves (1μg g^{-1}). Methanol was chosen as the desorption solvent, and the volume was 200μL. Several factors that affect the performance of custom-made fibers were optimized, including extraction and desorption time, desorption agitation speed and solvent pH.

3.4 In vivo SPME experiment

The in vivo SPME procedures were operated as follows:

In order to prevent the fragile quartz fiber from breaking, a medical hollow steel needle was first used to pierce the hard skin of the aloe plant and withdrawn, leaving a hole with a depth of about 1.5cm. The SPME fiber was then inserted into the hole the needle created. Make sure that the coating is fully covered by the cellulsic of the aloe in order to produce proper extraction. After a 20-min sampling, the SPME fiber was withdrawn from the leaf, rinsed with deionized water three times (60s each time) and dried with a Kimwipes.

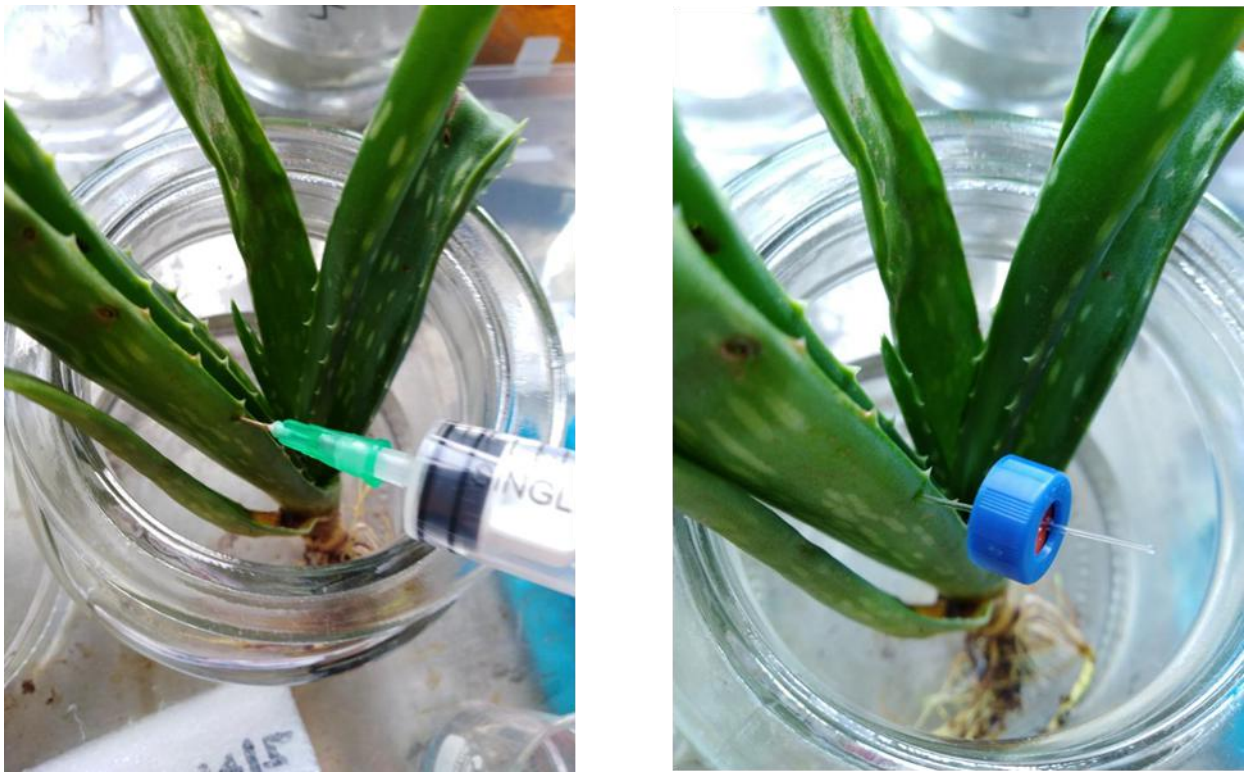


Figure 11 In vivo sampling from aloe by SPME fibers

Subsequently, the fiber was placed into a 2-mL vial containing 200ul HPLC-grade methanol and eluted for 60min on a rotator with 600rpm speed. Eluents were filtered through nylon filter membranes (pore size 0.45μm) and then introduced into LC-PAD determination.

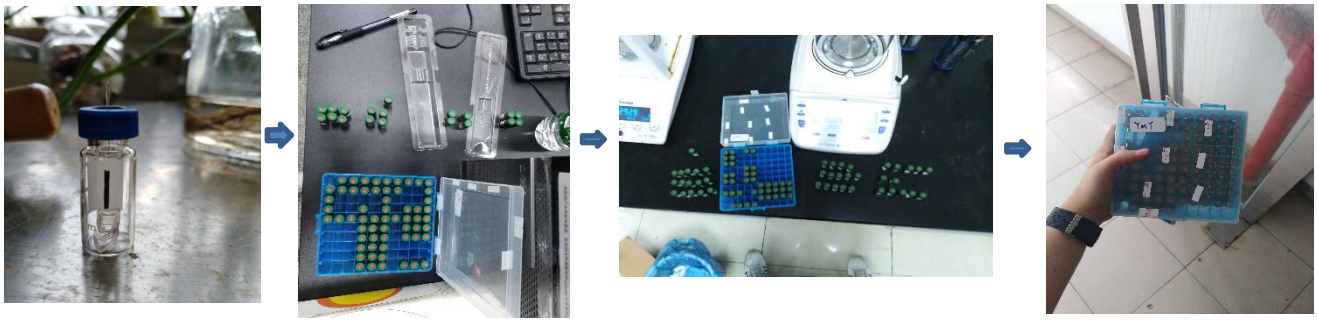


Figure 12 The extract is eluted from the SPME fibers for LC-PAD testing

3.5 Result and Discussion

3.5.1 Extraction performance of the custom-made fibers

(1) Intra-fiber and inter-fiber reproducibility

The reproducibility of the probe is an important indicator to verify the performance of the probe. In this experiment, the intra-fiber and inter-fiber reproducibility were investigated.

Using sample solution containing 1ppm SA and ASA, reproducibility of single needle was inspected by selecting a needle and continuously extract the sample solution 5 times to find the relative standard deviation of the area of 5 injection peaks (See Table 1); and the reproducibility between needles is inspected by selecting 5 needles to extract the sample solution separately and calculate the relative standard deviation of the 5 injection peak areas (See Table 2).

Table 1. Intra-fiber reproducibility (n=5) of custom made SPME fibers

SAs	RSD (%)
SA	8.45
ASA	4.42

Table 2. Inter-fiber reproducibility (n=5) of custom made SPME fibers

SAs	RSD (%)
SA	6.45
ASA	6.36

(2) Linear

The 20 ppm OPPs mixed solution was diluted stepwise to prepare

OPPs at concentrations of 5, 1, 0.5, 0.1, 0.05, and 0.01 ppm, respectively. Six SPME needles were used to sample 5 times at different concentrations, and the LC peak area-concentration was used as a linear regression curve (see Figure 13 and Table 3).

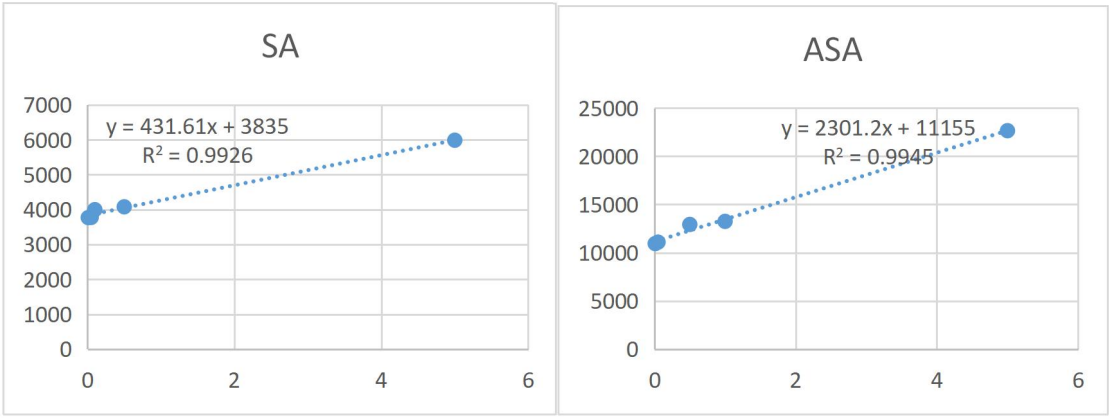


Figure 13 Linear regression curves of custom made SPME fibers

Table 3. Linear of custom made SPME fibers

SAs	Linear equation	R^2	Linearity (μgL-1)
SA	431.61x + 3835	0.9926	10-5000
ASA	2301.2x + 11155	0.9945	10-5000

3.5.2 In vivo tracing of SAs in aloe under microplastics stress

After in vivo extracted by SPME, the eluted samples were analyzed by HPLC System with photodiode array detector (LC-PAD). A series of LC spectra has been obtained. The typical LC spectrum are as follows (Figure 14). Peak at 2.4min is attribute to SA and 3.1min peak is ASA.

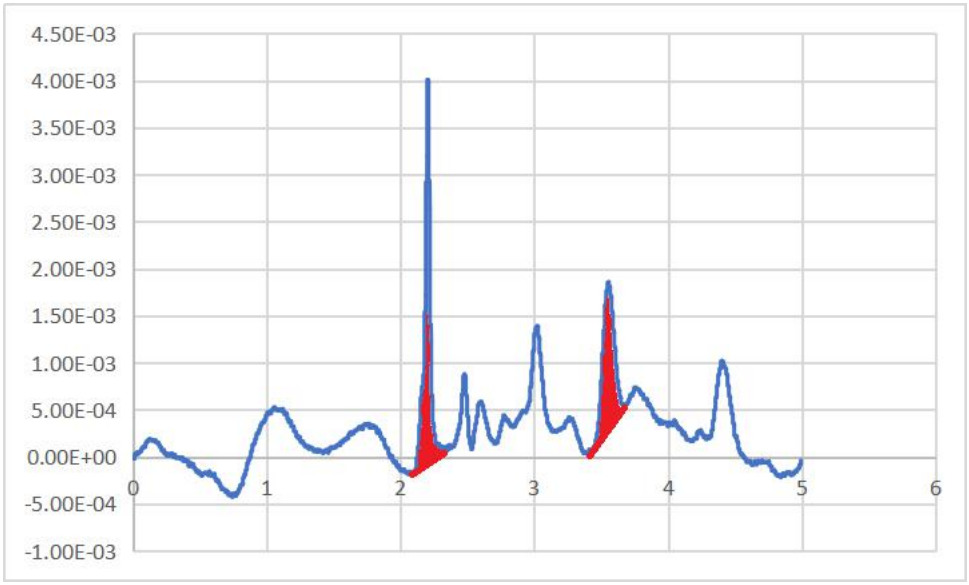


Figure 14 Typical spectrum of LC chromatography for testing SA and ASA in aloe

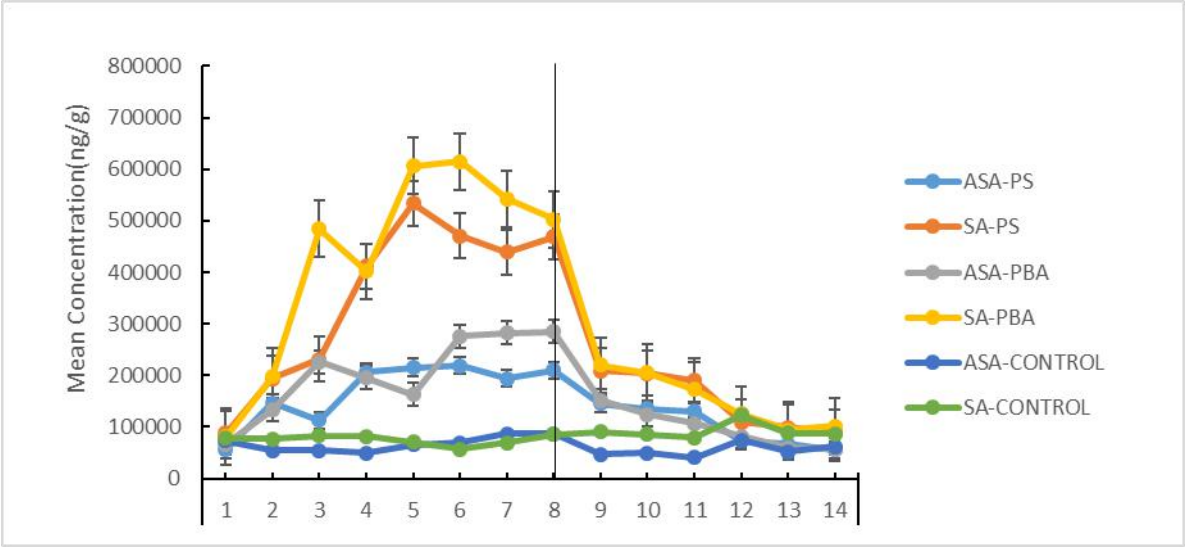


Figure 15 The concentration change of SA and ASA during exposure experiment under microplastics and for elimination without microplastics

Then the mean concentration of SA and ASA can be obtained by peak

area integral, and the concentration change of SA and ASA during exposure experiment are shown in Figure 15. A line is drawn in Figure 15 that marks the end of the exposure when the change in concentration reached balance, then the aloes were transferred to clear water containing no microplastics for elimination. It can be seen that the two SAs being tested all shown various degrees of increasing when subjected to the microplastic environment compared to control groups. SA amounts increased by 6.0 times and reached its peak at 5 to 6 days, while ASA amounts increased by 3.0 times and peaked at 4 to 6 days, then both shown slight decrease once reaching the peak. By the end of the eighth day, the SAs reaches a platform and the exposure section of the experiment ends. Aloes in the experimental groups are transferred to clear water. Once aloes are in clear water, both SA and ASA level show significant decrease. Previously published articles have revealed that both SA and ASA are involved in stress tolerance, and their contents will increase to activate the defense mechanisms, [19,23,32] which agreed well with the results obtained by our the in vivo tracing. These findings demonstrated the accuracy of the in vivo SPME method. Though SPME is renowned for its nonlethal sampling, small slits are still necessary for fiber insertion into the plant tissues. Studies found that aloes respond to the small slits by a slight increase of SAs, but the SAs contents returned to normal levels at 8–12h from the creation of the slit [29]. Therefore, it

can be reasoned that the upsurge of the stressed group was mainly caused by the bio-stress posed by the microplastics, and the injuries caused by fiber insertion had minor impacts on our experiments.

3.5.3 In vivo tracing of SAs in aloe under different concentration of microplastics

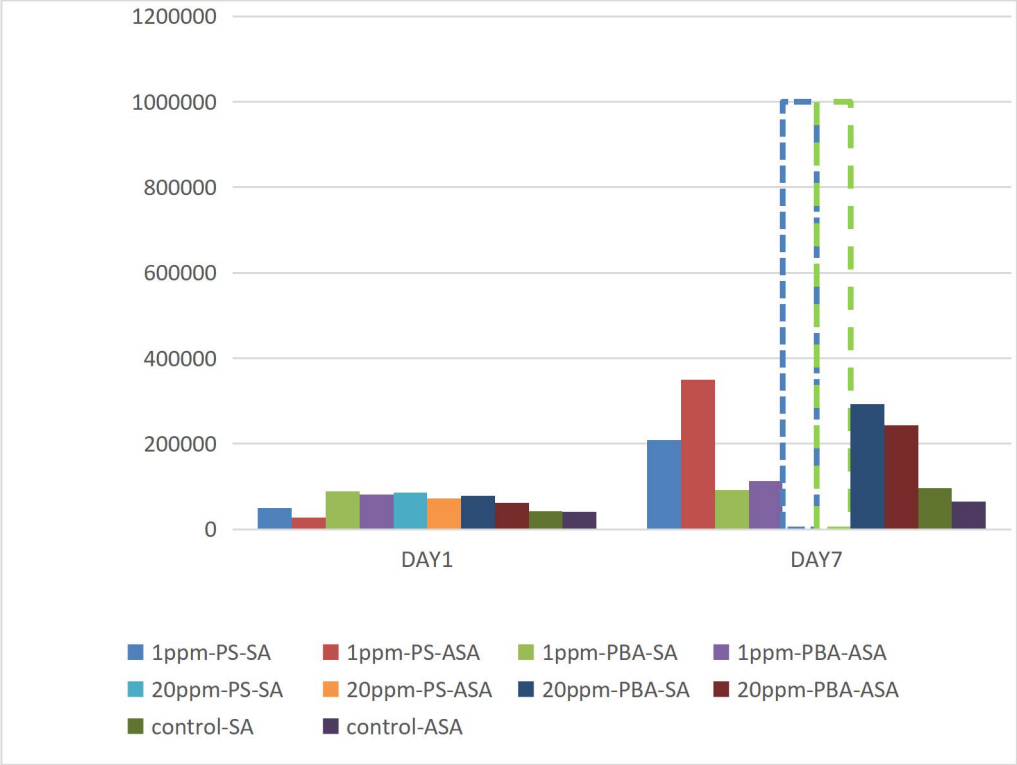


Figure 16 The SAs level under different concentration of microplastics. (Note: Columns in dashes shows that the signal has gone too high for LC machine to be detected)

A further study was conducted to investigate the effect different concentration of microplastics have on the SAs in a plant. The experiment separate aloes into 5 groups (1ppm of PS, 1ppm of PBA, 20ppm of PS, 20ppm of PBA, control group) with each froup containing three aloes. Samples was taken on the first day and the seventh day. Result shows that within similar concentration, PBA poses more stress on plants than PS. It

may ascribe this to size effect owing to PBA with larger size is more likely to clog plants tissue. Within similar microplastics, higher concentration poses more stress on plants.

3.6 Conclusion

In this work, C18@GO@PDDA composite was synthesized and applied for SPME coating materials, then a convenient and efficient in vivo SPME method was developed to determine and trace SA, ASA in aloe leaves, coupled with LC-PAD analysis. The in vivo SPME method featured low LODs and LOQs, wide linear ranges, as well as high accuracy and repeatability. The in vivo tracing of the targeted analytes in aloe leaves under microplastic stress showed that SAs, plant's regulator hormones, upsurged to activate the defense system against abiotic stress of microplastics. To the best of our knowledge, it was the first time that in vivo SPME technique was applied to trace the contents of SAs as plant regulators under microplastics stress. What's more, due to the nonlethal and simple operation, the result of the experiment identified microplastics as a source of bio-stress and discovered certain characteristics of microplastics as a pollutant.

4. In Vivo Tracing of The Effect of Microplastics on OPPs uptake and elimination in Aloe

4.1 Experimental Materials and Instruments

4.1.1 Chemicals and Materials

OPPs of Propetamphos, Quinalphos, and Profenofos were all purchased from Accustandard (New Haven, CT, USA). The materials mentioned above are not described here.

4.1.2 Analytical Instrument

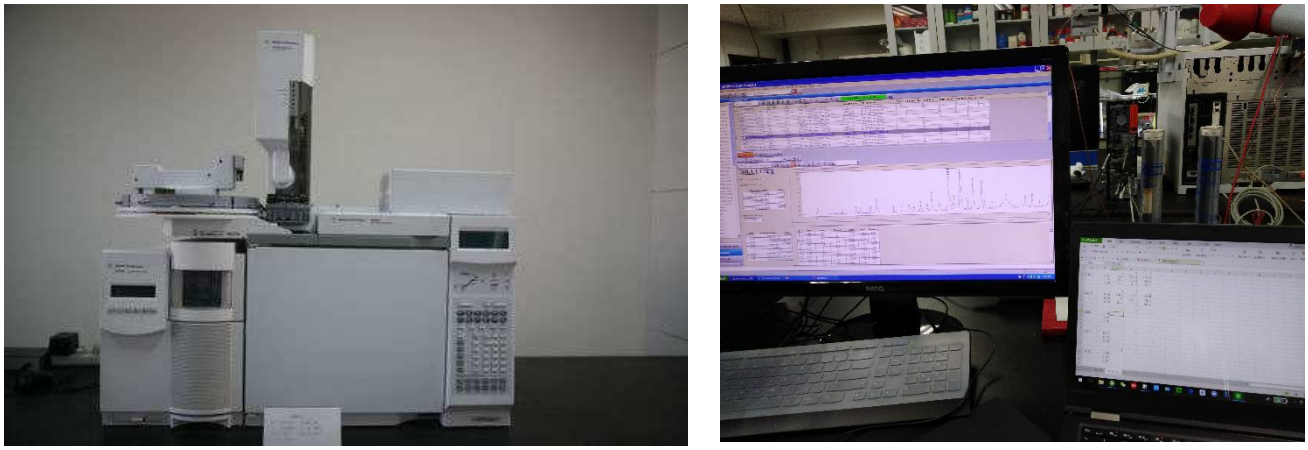


Figure 17 Gas chromatograph coupled to an mass spectrometer(GC/MS)

GC/MS analysis was performed on an Agilent 6890N gas chromatograph coupled to an Agilent 5975 mass spectrometer with an electron ionization (EI) source (Agilent Technologies, CA, USA). A HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies, CA, USA) was used for separation. Ultrapure helium was employed as the carrier gas. The inlet temperature was 250 °C, and the oven temperature programs were as follows. For OPPs, the column temperature was initially 100 °C for 2 min, ramped to 180 °C at a rate of 30 °C ·min−1, and held at 180 °C for 2 min, then increased to 200 °C at a rate of 3 °C·min−1, held at 200 °C for 4 min, increased to 250 °C at a rate

of 30 °C ·min−1, held for 3 min. The total run time was 26.7 min.[30]

4.2 Aloe exposure Experiment

All the aloe plants used were purchased from a local nursery. To imitate the effect of microplastics on the absorption of OPPs, aloe plants are cultivate in purified water to exclude influence of other factors. The water quality (pH, dissolved oxygen, and temperature) was monitored daily.

For the exposure experiment, one experimental groups(containing five aloes) are cultivated in 200ml of 20ppm PS and 1ppm of OPPs. Microplastics and OPPs are added simultaneously and sonicated for 20min before aloes are put into the solution.

Meanwhile, one control group (also containing five aloes) are cultivated in 200ml of distilled water with 1ppm of OPPs(also sonicated for 20min before aloes are put in the solution). Samples are taken daily at 11am.

After the uptake tracing period which lasted for 5 days, aloes are taken out of the spiked solution and transferred to distilled water after their roots are washed.

4.3 In vivo SPME Experiment

The in vivo SPME procedures of extraction OPPs from aloes were operated as follows.

The organ tissues was pierced with a 26 gauge hypodermic needle to a depth of about 1.5 cm. Each needle extract from one aloe

and only the leaf part of the aloe is tested.

The SPME fiber was then inserted into the hole the needle created. Make sure that the coating is fully covered by the cellulsic of the aloe in order to produce proper extraction. After a 15-min sampling, the SPME fiber was withdrawn from the leaf rinsed with deionized water three times (60s each time) and dried with a Kimwipes. Subsequently, the fiber was placed into a GC to determine the intensity signals for the OPPs without elution.

4.4 Result and Discussion

4.4.1 Extraction performance of the PDMS fiber

(1) Intra-fiber and inter-fiber reproducibility

In this experiment, the intra-fiber and inter-fiber reproducibility were investigated. Using sample solution containing 1ppm OPPs, reproducibility of single needle was inspected by selecting a needle and continuously extract the sample solution 5 times to find the relative standard deviation of the area of 5 injection peaks (See Table 4); and the reproducibility between needles is inspected by selecting 5 needles to extract the sample solution separately and calculate the relative standard deviation of the 5 injection peak areas (see Table 5).

Table 4 Intra-fiber reproducibility (n=5) of PDMS fibers

OPPs	RSD (%)

Propetamphos	8.34
Quinalphos	25.00
Profenofos	23.14

Table 5 Inter-fiber reproducibility (n=5) of PDMS fiber

OPPs	RSD (%)
Propetamphos	10.22
Quinalphos	16.19
Profenofos	21.04

(2) Linear

The aloe cellulsic was mixed with OPPs at concentrations of 5, 1, 0.5, 0.1, 0.05, and 0.01 ppm, respectively. Three SPME needles were used to sample every different concentration, and the GC peak area-concentration was used as a linear regression curve (see Figure 18).

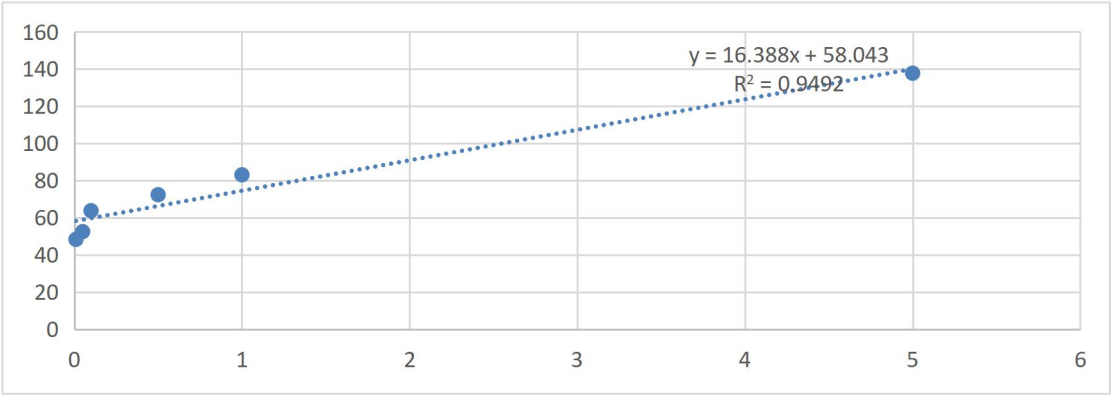


Figure 18 Concentration linear regression curve of PDMS fibers

Extraction time was optimized by creating an extraction curve using the GC peak area-concentration. The aloe cellulsic was mixed with 1ppm

OPPs and 3 needles were used to test every different extraction time which are 5, 10, 20, 30, 50 and 70min. The GC peak area-concentration was used as a linear regression curve (see Figure 19).

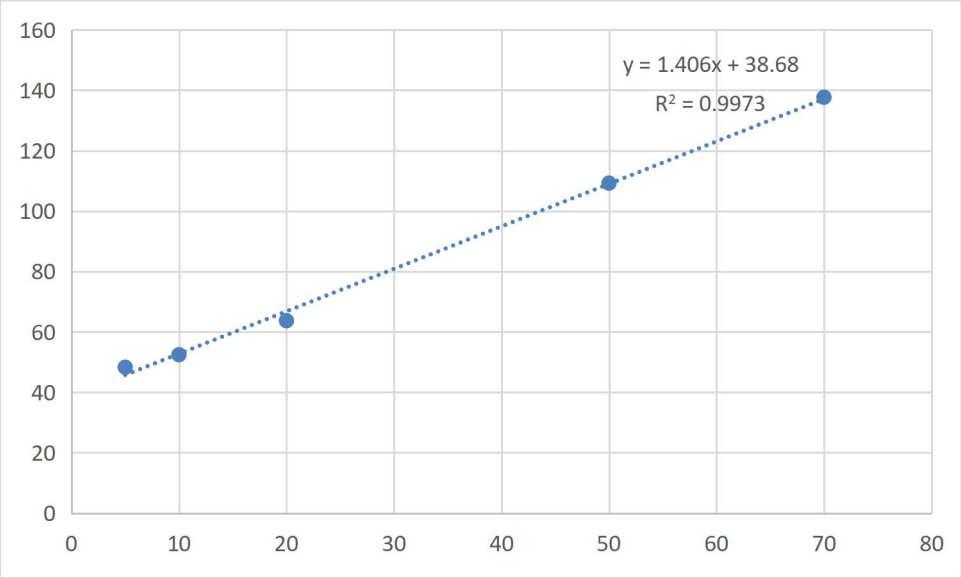


Figure 19 Concentration linear regression curve of PDMS fibers

4.4.2 In vivo tracing of OPPs uptake in aloe under microplastics stress

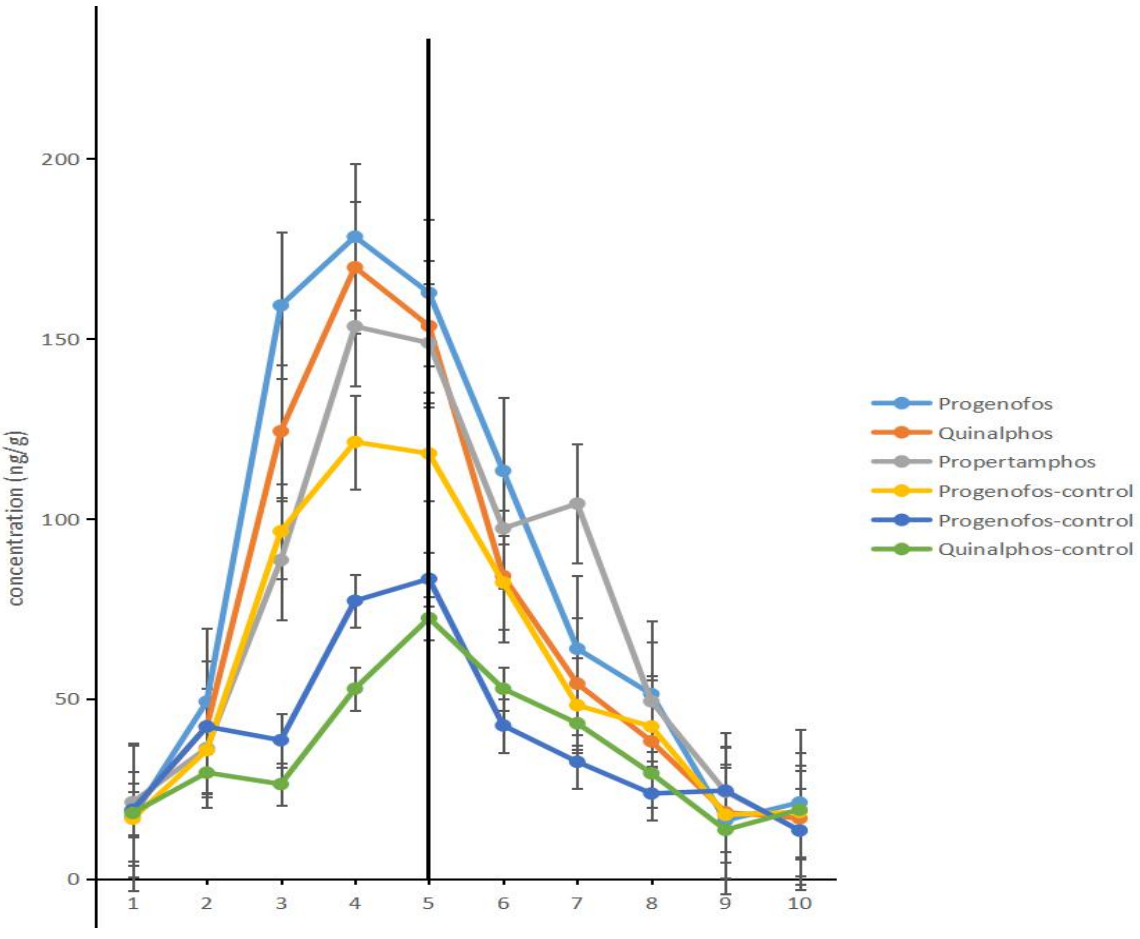


Figure 20 The concentration change of OPPs during exposure experiment under microplastics and for elimination without microplastics

The OPPs being extracted from aloes in the experimental group all shown higher signal than the OPPs extracted form aloes in the control group. A line is drawn that marks the end of the exposure and all aloes are transferred to distilled water containing no microplastics nor OPPs. Compared with the control group in which aloes are only exposed to OPPs, the OPPs signal is higher in the experimental group in which aloes are exposed to both microplastics/OPPs. OPPs absorption reaches its peak at around 4 to 5 days, and all shown slight variation.

The results obtained by this vivo tracing SPME of living plant revealed that microplastics would increase the rate OPPs access plants, agreed well with previously published articles on effect of nanoparticles.[33] This finding demonstrated that microplastics can increase the rate OPPs access the plants owing to surface adsorption and enrichment effect. The biotic stress of microplastics accelerate the OPPs uptake in aloe.

4.5 Conclusion

In this work, custom made PDMS fibers was applied for SPME, then a convenient and efficient in vivo SPME method was developed to determine and trace organic pollution of OPPs in aloe leaves, coupled

with GC-MS analysis. The in vivo tracing of the targeted analytes, OPPs, in aloe leaves under microplastic adsorption effect shows that microplastics can increase the rate OPPs access plants and more OPPs uptake in aloe under microplastics stress. To the best of our knowledge, it was the first time that the adsorption effect of microplastics on the rate of OPPs uptake in plants is tested. What's more, due to SPME technology, the result of the experiment identified microplastics as a source of pollutant that can poses serious harms when combined with OPPs.

5. Conclusion and Significance

In this research, a in vivo SPME bio-sampling technique is put forward to trace and monitor the dynamic change of uptake, accumulation, elimination and residue behavior of endogenous phytohormones and organpollution in plants under the influence of micro-plastic pollution. The mechanism of the influence of microplastics pollution on plant growth is explored.

C18@GO@PDDA composite was synthesized and applied for SPME coating materials, then a convenient and efficient in vivo SPME method was developed to determine and trace SA, ASA in aloe leaves, coupled with LC-PAD analysis. The in vivo SPME method featured low LODs and LOQs, wide linear ranges, as well as high accuracy and repeatability. The in vivo tracing of the targeted analytes in aloe leaves under

microplastic stress showed that SAs, plant's regulator hormones, upsurged to activate the defense system against abiotic stress of microplastics.

Custom made PDMS fibers was applied for SPME, then a convenient and efficient in vivo SPME method was developed to determine and trace organic pollution of OPPs in aloe leaves, coupled with GC-MS analysis. The in vivo tracing of the targeted analytes, OPPs, in aloe leaves under microplastic adsorption effect shows that microplastics can increase the rate OPPs access plants and more OPPs uptake in aloe under microplastics stress.

Up to now, systematic studies on the effects of microplastics on plant growth has not been reported. To the best of our knowledge, it was the first time that in vivo SPME technique was applied to trace the contents of SAs as plant regulators under microplastics stress and the adsorption effect of microplastics on the rate of OPPs uptake in plants. What's more, due to the nonlethal and simple operation, the result of the experiment identified microplastics as a source of bio-stress and discovered certain characteristics of microplastics as a pollutant.

Carrying out this research has important theoretical and practical significance for comprehensive evaluation of the biological effects of soil microplastic pollution, for assessment of environmental risks and human health risks of microplastics.

6. "Clean Soil"-----A Research Proposal on enzyme degradation of microplastics

It is not an uncommon sight to see beverage bottles, food wrapping, and fibers used in clothes everywhere, forming “network” of microplastics pollution. Monster of microplastics has been released from the bottle. Do we just stand by?

Biological degradation of microplastics may be one solution for clean soil. Rare examples include a new thermophilic hydrolase from Thermobifida fusca that can degrade microplastics; [33] and one famous study done by Japanese scientists on isolating Ideonella sakaiensis 201-F6, a novel bacteria that can use microplastics as its major energy and food source that produce two enzymes which can break down molecular chain of plastics. [34]

Because the ability to enzymatically degrade microplastics has been thought to be limited to a few fungal species, biodegradation is not yet a viable remediation or recycling strategy. Also, organism is strongly effected by environmental factors, which means different environment can effect organisms to evolve differently, creating the type of organism that is most suitable for degrading plastics in that specific environment. To tackle a local plastics problem, it is better to start by studying local enzyme capable for degrading microplastics.

To solve this problem we will try to find the most effective enzyme in local sample by firstly collect local water or landfill sample and cultivate organisms (fungi and bacteria) on microplastics as their only source of carbon, secondly identify organisms capable of degrading plastics, thirdly identify enzymes involved in degrading plastics, and lastly compare the effectivity of the newly found enzyme with the currently proven most effective enzyme. The feasibility of my proposed method is supported by existing articles and natural evolution.

The hypothesis is that the microorganisms that thrives in places where microplastics exist longer will evolve to use microplastics as a source of their energy, therefore, some microorganisms might possess the ability to degrade microplastics.

Thus, the targeting biocatalyst is expected to be some enzymes extracted from one or different biological organism that is only found in local environment and is capable of degrading microplastics. The improved biocatalyst is aimed to provide the most effective way to degrade local microplastics since it has already adapt to the local environment.

Aim 1 will isolate the enzyme that can degrade microplastics by sampling from Liuxi River and farmland in Zhongluotan District and cultivate organisms (fungi and bacteria) on microplastics as their only source of carbon. Then testify the efficiency of the identified

microorganism. If there is no microorganisms that really stand out in terms of its special ways of degrading microplastics, choose the most effective one and identify the enzyme by characerizing its DNA structure, compare that with the DNA database to find out which group the microorganisms is in. Lastly, compare the new microorganisms found in the Zhongluotan District with the current most effective bacteria found by the Japanese scientist to see which is more effective at degradation plastics in the Zhongluotan water and soil sample.

Aim 2 will focus on the enzyme that added the degradation of microplastics by testify which is doing the degrading job and compare the activity of the found protein with that of three evolutionarily divergent enzymes of the same class to see their activity on microplastics.

If characterized and identified, the organism of the enzyme involved in degrading plastics will serve as an environmental remediation strategy when applied in nature. Also, the microorganisms with the enzymatic machinery can serve as a degradation platform for biologically recycling of microplastics pollution.

What's more, if proven that in the new microorganisms found in local sample is more effective than the currently most effective bacteria found by the Japanese scientist, the model can be implemented in more places in tackling local microplastics pollution with local microorganisms.

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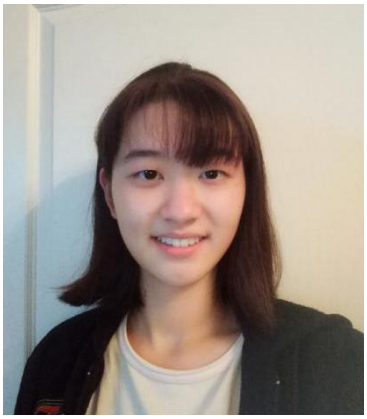
ACKNOWLEDGMENTS

Resume of Team Member



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Research Interests:

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