Comparative Studies on Effects of Acid Solutions on Aquatic Plants by Beam Deflection and Absorbance Spectroscopy Methods

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The beam deflection method and absorbance spectroscopy were applied to study effects of acid solutions on aquatic plants, and their results were compared. Aquatic plants Egeria densa and Ceratophyllum demersum L were used as model plants. In absorbance experiments, a piece of the plants was put in a beaker with 20 mL HCl solution, and absorbance of the HCl solution was measured every 30 min. In beam deflection experiments, a probe beam from a He-Ne laser was focused to a vicinity of the plants in a culture dish with HCl solution by an objective lens, and deflection signals of the probe beam were monitored by a position sensor. Absorbance spectra of the HCl solutions with immersing of the plants showed absorbance below 410 nm, suggesting that some compounds leaked from the plants into the HCl solutions. Changes of absorbance and deflection signals with immersion time were examined for different pH levels. The changing trends of the absorbance and deflection signals with time were similar, but the absorbance changes were delayed for about 2 - 3 h. The absorbance method could not detect the effect of the pH 5.0 HCl solutions on the aquatic plants, while the deflection method could.

Keywords Deflection, probe beam, absorption spectroscopy, HCl solutions, aquatic plants

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Introduction

Acid rain and acid solutions can cause many changes in terms of both biochemistry and physiology on plants including changes of morphology and anatomy, respiration and photosynthetic rate, and concentrations of components in the plants.1,2 Many analytical methods have been used for studying the impact of acid rain or acid solutions on plants. For example, atomic absorption spectroscopy was used for the determination of minerals in plants treated with acid rain.3 Recently, we have applied the deflection method of a probe beam for optical sensing of material movements at different locations of a plant.4 Deflection signal of the probe beam passing through a vicinity of the plant was caused by change of concentration gradients of chemical species involved in the material movements across the plant surface. Furthermore, the deflection method was applied for studying the effects of acid solutions on both terrestrial and aquatic plants.5

Although the deflection method could be used for noninvasive sensing of material movements across the plant surface, it could not detect what kinds of materials leaked from the plants or were taken in the plants. Also, the quantitative evaluation of the material movements across the plant surface was not easy, because the deflection signal was induced by the sum of changes of concentration gradients of all compounds involved. In order to obtain more details about the material movements across the plant surface, the deflection method has to be combined with other analytical methods. Here, the deflection method and absorbance spectroscopy are applied to study the effects of acid solutions on aquatic plants simultaneously, and their results are compared. Aquatic plants Egeria densa and Ceratophyllum demersum L were used as the model plants.

Experimental

Figure 1 shows the experimental setup for the deflection method and photos of the model plants. The experimental setup was similar to that reported earlier.5 A short piece of Egeria densa or Ceratophyllum demersum L (about 0.3 cm long) was put in a culture dish with 20 mL HCl solution of a certain pH, or distilled water. The culture dish was put on the stage of a microscope. A He-Ne laser (output power: 3 mW; wavelength: 633 nm) was used as a light source of the probe beam. The probe beam was focused (spot size: about 3 - 5 μm) on the plant/water interface in the culture dish by an objective lens (NA: 0.1). An X-Y micro-stage was used to adjust the distance between the laser beam and the plant surface. The distance between the laser beam center and the plant surface was about 2 - 3 μm. The deflected probe beam was reflected by a mirror, and then it was focused to a position sensor by a lens. A filter (Toshiba Glass Co., Ltd., R-62), which cut off light shorter than 610 nm, was placed in the front of a position sensor to decrease background noise. Transmittance of the filter at 632.8 nm was about 80%.

In the absorbance experiment, a spectrophotometer (U-2910,
Hitachi) was used. A piece of the plants (about 6 cm long) was entirely immersed in 20 mL HCl solution with a certain pH in a 30 mL beaker. Absorbance spectrum of the HCl solution in the beaker was measured about every 30 min, and the corresponding HCl solution without immersion of the plants was used as a reference. A pH 1.0 HCl solution was prepared by adding hydrochloric acid (Wako Pure Chemical Industries, Japan) to distilled deionized water until pH 1.0 in a 300 mL beaker, where a pH meter (Horiba Ltd., F-51) was immersed for monitoring pH. A pH 2.0 HCl solution was prepared by adding the pH 1.0 HCl solution to distilled deionized water until pH 2.0. Similarly, HCl solutions of pH 3.0, 4.0 and 5.0 were prepared.

Results and Discussion

Figure 2A shows an absorbance spectrum of the pH 2.0 HCl solution after immersion of *Egeria densa* for about 6 h. The spectrum indicated that some compounds with light absorbance below 410 nm were leaked from the specimen and dissolved in the HCl solution. Although leaching of nutrients and foliar calcium from plant leaves by acid solution and acid rain has been reported, identification of the leaking compounds from *Egeria densa* by the HCl solution has not been reported as far as we know. On the other hand, peaks at about 470, 625, 646, 664, and 730 nm, which were characteristic absorbance peaks of pigments such as chl *a*, chl *b*, and carotenoids, were not observed. Therefore, Fig. 2A indicated that the leaked compounds by the HCl acid solution were not plant pigments.

Figure 2B shows the absorbance changes with time at 245, 320, and 410 nm with immersion. It is clear that the absorbance increased with time, especially after about 60 min, the absorbance increased fast with time, then became slow after about 240 min. Since the absorbance is proportional to concentrations of the leaked compounds in the HCl solution, Fig. 2B indicates that concentrations of the leaked compounds in the HCl solution increased with time.

Figure 2C shows deflection signals in a vicinity of the *Egeria*
Changes of absorbance and deflection signals with immersion time were also investigated in HCl solutions of pH 1.0, 3.0, 4.0 and 5.0. It was found that the lower pH of the HCl solution, the larger the changes in absorbance and deflection signals. When the *Egeria densa* was immersed in the pH 3.0 HCl solution, the absorbance was much smaller than those at pH 1.0 and 2.0. This meant that amounts of the leaked compounds in pH 3.0 HCl solution were much smaller than those in pH 1.0 and 2.0. When the *Egeria densa* was immersed in HCl solution with pH 4.0, absorbance of the HCl solution after 4 h of immersion were on or below the detection limit of the absorbance spectrometer. This suggested that the leaked compounds in the pH 4.0 HCl solution werefew. When the *Egeria densa* was immersed in the HCl solution with pH 5.0, no absorbance below 410 nm was detected after 4 h of immersion. This meant that the leaked compounds by the pH 5.0 HCl solution were not detectable by the spectroscopic method. It should be noted that the pH of the above HCl solutions after immersion of the *Egeria densa* would be changed because of the leaking of the compounds.

Figure 3 shows changes of the deflection signals with time at the vicinity of the *Egeria densa* in the pH 5.0 HCl solution and distilled water. The difference between the maximum and minimum of the deflection signals in monitoring periods of 1000 s has been used as a measure of changes of the deflection signals at the vicinity of a plant4 and a cell.9 Here, the difference (ΔS) between the maximum and minimum of the deflection signals in monitoring periods of 150 min was compared as shown in Fig. 3. Values of ΔS were 0.024 and 0.007 mm for the pH 5.0 HCl solution and distilled water, respectively. It is clear that changes of the deflection signals in the pH 5.0 HCl solution was larger than that in the distilled water. This suggested that leaking of the compounds by the pH 5.0 HCl solution might still have occurred. Therefore, for monitoring the leaking of the compounds from the plants by the acid solution, the beam deflection method is more sensitive than the absorbance one.

Effects of the HCl solutions on *Ceratophyllum demersum* L were also studied by the absorbance and deflection methods, and similar results were obtained.

As a conclusion, comparative studies on effects of HCl solutions on aquatic plants by the deflection and absorbance spectroscopy methods were carried out. In comparison with the deflection signals, the absorbance changes with time were delayed for about 2 – 3 h due to the diffusion from the plant surface to the whole HCl bulk solutions. The absorption spectroscopy method could detect the effect of the HCl solution below pH 4.0, while the deflection method could do so even at pH 5.0. Analysis of the HCl solutions after immersing of the aquatic plant, including change of pH and identification of the leaked compounds, remains to be studied.
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