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Attachment of *Cryptosporidium*-Sized Microspheres by Holly Plant Roots

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ABSTRACT

In order to prevent the outbreak of cryptosporidiosis, understanding the pathogenic parasite *Cryptosporidium* transport mechanism in the surface water body is critical. *Cryptosporidium* transport from soil to surrounding water bodies is a main source of pollution and impacted with the attachment by soil and plants. However, the attachment of *Cryptosporidium* by soil or plants is not clear. In this study, *Cryptosporidium* attachment by holly plant (*Ilex* spp.) roots and the impact factors were studied. Results showed that holly plant roots could attach the *Cryptosporidium*-sized microspheres and low soil pH and high sodium ion intensity can promote the attachment. But high-level *Cryptosporidium*-sized microspheres lead to the decreased attachment. It concluded that holly roots reduced the migration of *Cryptosporidium* due to the attachment.

INTRODUCTION

Cryptosporidium is a pathogenic parasite. As little as 10 *Cryptosporidium* oocysts can cause human disease, cryptosporidiosis. Watery diarrhoea, dehydration, fever, vomiting, physical weakness and abdominal pain are the main clinical manifestation. Infants and people with weak immunity can die due to cryptosporidiosis.

Soil and sediment is the sink of *Cryptosporidium*. Researches show that soil and sediment contained *Cryptosporidium* can migrate to surrounding water bodies by runoff (Harter et al. 2000, Xu 2016). However, the migration is controlled by multiple factors, for example, attachment by soil, attachment by plant roots, soil physical and chemical parameters, runoff intensity, runoff duration, etc. The objective of this paper is to study the attachment of *Cryptosporidium* by soil as well as the plant roots, in order to help first evaluate the *Cryptosporidium* migration under different soil conditions.

MATERIALS AND METHODS

Experimental materials: According to past research experiences, *Cryptosporidium*-sized microspheres were used, made of polystyrene (purchased from Polysciences, USA) (Lu et al. 2017a, 2017b, Lu & Amburgey 2016). The number of samples were examined under a fluorescence microscope. The root system of the plant in the experiment was taken from the roots of holly, which is the common landscape greening plant in Xuzhou area, and its roots are denser and have better control on the length and diameter . The roots of each experiment were kept similar to each other to reduce the experimental error.

Experimental methods: Attachment experiments were carried out using 250 mL Erlenmeyer flask and oscillating device. First, the pretreatment of ordinary landscape planted soil was done in the campus by taking 50 g soil, crushing and sieving it through 80 mesh sieve. It was added later into a 250 mL Erlenmeyer flask. The roots of holly was taken into the conical flask and 150 mL of tap water was added. The control parameters included the pH value of the solution (pH=5, 7 and 9), sodium ion strength (0.1 mol/L, 0.2 mol/L, 0.3 mol/L), and *Cryptosporidium* oocysts initial concentration (10^4 microspheres/mL, 5×10^4 microspheres/mL, 10^5 microspheres/mL).

RESULTS AND DISCUSSION

Attachment by soil combined with plant roots: Based on the experimental group (attachment by soil and plant root) and the control group (attachment by soil), the attachment of *Cryptosporidium* oocysts is shown in Fig. 1. The results showed that the amount desorption of *Cryptosporidium* oocysts in the experimental group was lower than that in the control group, which indicated that the average number of adsorbed *Cryptosporidium*-sized microspheres with plant roots is more than that by the soil only under different soil physical and chemical properties. The root system of the

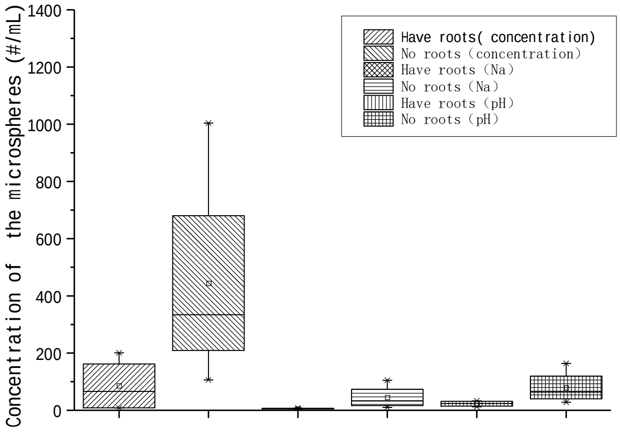

Fig. 1: The variety of the number of *Cryptosporidium* oocysts in the water samples of each experimental group (the experimental variables included the initial concentration of *Cryptosporidium* oocysts substitution, Na^+ concentration and soil pH).

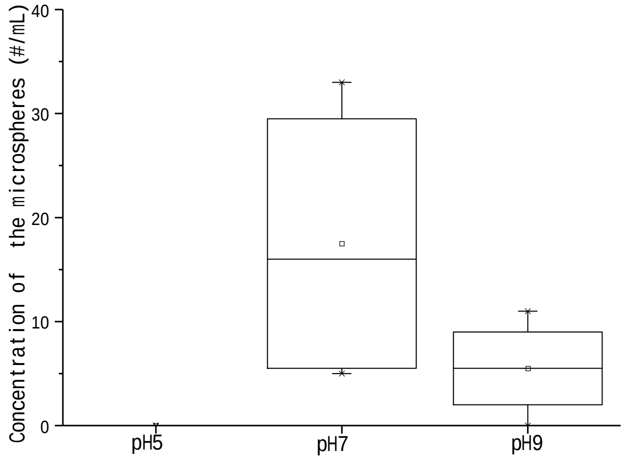

Fig. 2: The total release of *Cryptosporidium* oocysts substitute under different pH conditions (experimental group data).

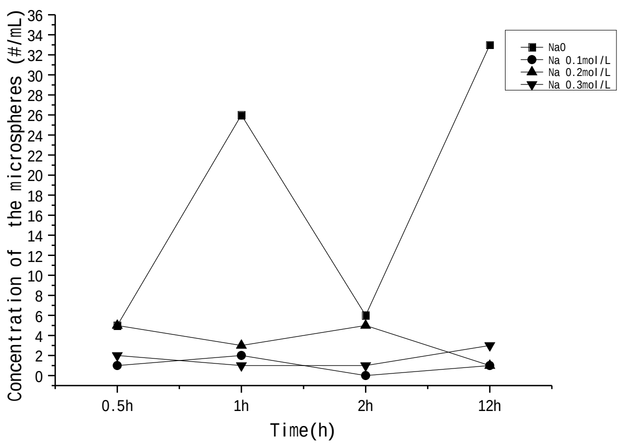

Fig. 3: Variety of the release of *Cryptosporidium* oocysts substitute with time under different pH conditions (experimental group data).

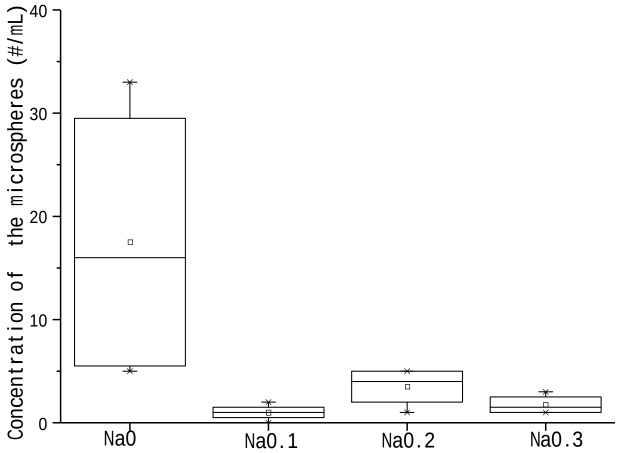

Fig. 4: The total release of *Cryptosporidium* oocysts substitute under different Na^+ concentration conditions (experimental group data).

holly has a significant attachment effect on the *Cryptosporidium*-sized microspheres. It was because the plant root surface provides a large number of binding sites for the *Cryptosporidium*-sized microspheres. The *Cryptosporidium*-sized microspheres were adsorbed on the surface of the plant roots, thereby reducing the migration of *Cryptosporidium*-sized microspheres from the soil and root system to the surrounding water. The *Cryptosporidium*-sized microspheres have a particle size of 4-6 μm, and their attachment mechanisms are similar to those of colloid. The colloid research showed that dense vegetation can effectively intercept surface runoff contaminants (Yu et al. 2013).

Effects of soil pH on the attachment: Fig. 2. shows the total desorption of *Cryptosporidium*-sized microspheres at different pH values. Figs. 2 & 3 show the desorption of *Cryptosporidium*-sized microspheres varied over time at different pH values. When pH value was 5, the attachment of *Cryptosporidium*-sized microspheres is obvious, and the number of *Cryptosporidium*-sized microspheres detected in water samples was little, which was significantly lower than that at the pH value of 7 and 9. Fig. 3 shows that at the beginning of the experiment, the attachment and desorption behaviour of the oocysts were not balanced at the beginning. The DLVO theory suggests that the attachment capacity of colloids is generally poor at high pH because of the increase in electrostatic repulsion and the inhibition of colloidal attachment at high pH values. On the other side, low pH can enhance the attachment of colloids (Zheng 2016). These results agreed that the lower pH value could significantly enhance

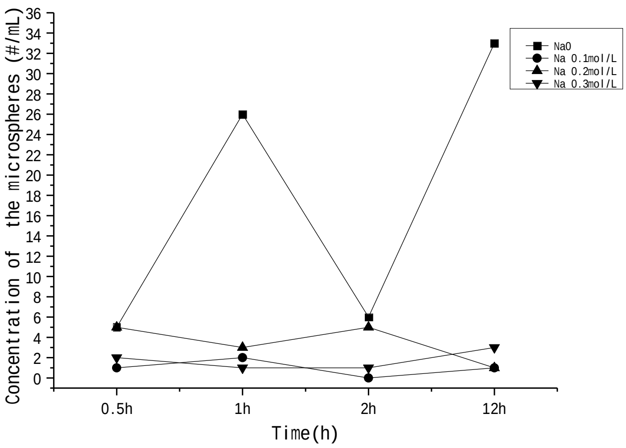

Fig. 5: Variety of the release of *Cryptosporidium* oocysts substitute with time under different Na^+ concentration conditions (experimental group data).

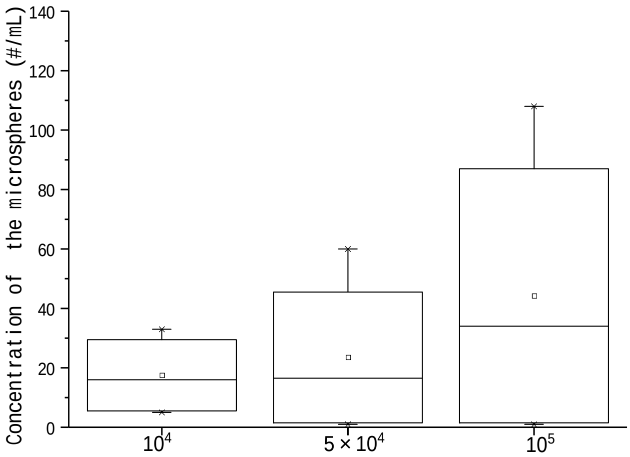

Fig. 6: The total release of *Cryptosporidium* oocysts substitute under the different initial concentration of substitute (experimental group data).

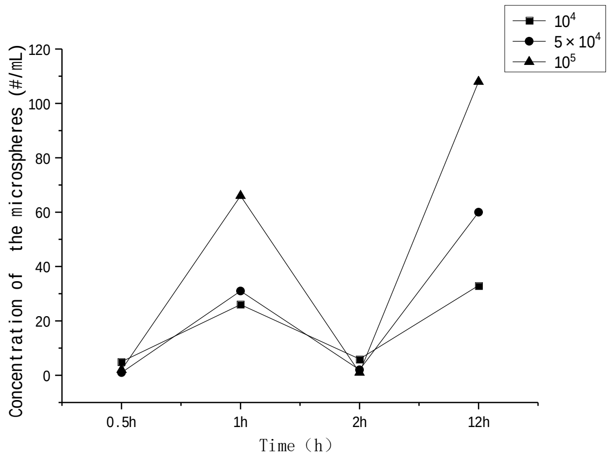

Fig. 7: Variety of the release of *Cryptosporidium* oocysts substitute with time under different initial concentration of substitute (experimental group data).

the attachment of *Cryptosporidium*-sized microspheres, which affected the migration of *Cryptosporidium*-sized microspheres.

Effects of soil Na^+ intensity on the attachment: Fig. 4 shows the total desorption of *Cryptosporidium*-sized microspheres in the experimental group under different sodium ion intensity conditions. Fig. 5. shows the change of the release of *Cryptosporidium*-sized microspheres in the experimental group with time under different sodium ion intensity. Results showed that the sodium ion strength has a great effect on the attachment and desorption of *Cryptosporidium*-sized microspheres. With the increase of sodium ion intensity, the desorption number of *Cryptosporidium*-sized microspheres in water samples decreased significantly. The number of *Cryptosporidium*-sized microspheres in the water samples fluctuate, but when the sodium ion intensity was 0, the number of *Cryptosporidium*-sized microspheres is highest at each time point. After 12 h, the attachment and desorption reactions were basically balanced. The water samples were collected for 12 hours, and the results showed that the desorption number of *Cryptosporidium*-sized microspheres decreased gradually with the increase of sodium ion intensity. The surface of *Cryptosporidium*-sized microspheres has a negative charge and the charge is about -30 mV (Lu 2012). The increase of sodium ion enhances the attachment of *Cryptosporidium*-sized microspheres by plant roots and soils and reduces the migration of *Cryptosporidium*-sized microspheres from soil and root system to water bodies. The underlying reason is that the higher sodium ion compresses the double electron layer on the surface of the charged material. Zevi et al. (2009) studied the colloid migration in porous media, suggesting that the high ionic strength of the solution reduces the repulsive force between the colloid and the surface of the medium, thereby promoting colloid deposition to the surrounding surface of the medium, which is consistent with the results of this study.

Effects of initial concentration of *Cryptosporidium*-sized microspheres on its attachment: Fig. 6. shows the total release of *Cryptosporidium*-sized microspheres in the experimental group at different initial *Cryptosporidium*-sized microsphere concentrations. Fig. 7 shows the release of *Cryptosporidium*-sized microspheres with time. It can be seen from Fig. 6 that the higher the initial concentration of *Cryptosporidium*-sized microspheres, the more the number of *Cryptosporidium*-sized microspheres in water samples. It can be seen from Fig. 7 that the number of *Cryptosporidium*-sized microspheres in the water sample fluctuates with time, and after 12 hours, the released number of *Cryptosporidium*-sized microspheres in the experimental group was stable. Meanwhile, the total number of *Cryptosporidium*-sized microspheres in the water samples increased with the increase of the initial dosage.

CONCLUSION

The attachment of *Cryptosporidium*-sized microspheres by soil and roots was studied under different soil physical and chemical conditions. The following conclusions were made:

1. Holly roots have a certain attachment effect on *Cryptosporidium*-sized microspheres, which is to inhibit the migration of *Cryptosporidium*-sized microspheres from soil to the surrounding water.
2. Low soil pH can promote the attachment by holly root to the *Cryptosporidium*-sized microspheres.
3. High sodium ion intensity can promote the attachment by holly root to the *Cryptosporidium*-sized microspheres.
4. *Cryptosporidium*-sized microspheres release increased with the initial concentration.

Therefore, holly roots, lower soil pH, and high sodium ion intensity can help reduce the migration of *Cryptosporidium* due to the attachment by soil and holly roots.

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