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SARS-CoV-2 and other pathogens could be determined in liquid samples from soils☆

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A B S T R A C T

In the current COVID-19 pandemic, SARS-CoV-2 has been quantified in wastewater in various countries, and wastewater based epidemiology has been proposed as a potential early warning tool for new outbreaks. However, even taking into account that poorly treated wastewater and sewage sludge may be spread on soils, there is no published paper dealing with the quantification of the virus in soil-related liquid samples, as could be runoff, leachates, or soil solution. To fill this gap, the authors of this piece propose reflections on the development of a methodological approach for the quantification of SARS-CoV-2 (and eventually other pathogens) in soil-related liquid samples.

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1. Why studying SARS-CoV-2 and other coronavirus (or other pathogens) in soil-related liquid samples?

Coronaviruses suffer continuous mutations, increasing risks of zoonotic transmission of diseases, and making easier that eventual new coronaviruses cause future outbreaks (Carducci et al., 2020; Daszak et al., 2020; Ye et al., 2020), as happens with other viruses (Gerba et al., 2017).

As indicated by authors such as Carducci et al. (2020), Heller et al. (2020), Hindson (2020), McCall et al. (2020), or Thompson et al. (2020), the fecal-oral transmission route should be considered as a possibility for SARS-CoV-2, as for other pathogenic microorganisms. Moreover, authors such as Lyon and Wang (2012), Longdon et al. (2014) or Dawood (2020) stated that new potential routes and characteristics for transmission should be considered for microbes that suffer frequent mutations.

Previous papers (for example Daughton; 2020; Farkas et al., 2020a; Kitajima et al., 2020; Race et al., 2020; Sims et al., 2020) have suggested that wastewater based epidemiology (WBE) could be useful to report on the incidence of COVID-19, and as early warning tool. In addition, Núñez-Delgado (2020a) commented on eventual effects to be considered in different environmental compartments, including soils affected by sewage sludge spreading or being irrigated with poorly treated wastewater. In fact, Gerba et al. (2017) have previously indicated that there is a need for risks assessment and frequent updating as regards viruses contained in wastewater recycled, specifically when used for irrigation purposes.

With a precautionary view, the assessment should be also expanded to water bodies (surface and groundwater) and plants growing in the area (Carducci et al., 2020; Núñez-Delgado, 2020a), mostly in locations where wastewater and sludge are not treated. At these places, additional care should be taken where aerosols containing microbes could be generated (Kitajima et al., 2020; Nghiem et al., 2020). Surface and subsurface runoff, as well as lixiviates or leachates generated in areas receiving the spreading of sewage sludge and wastewater could transfer SARS-CoV-2 (or other pathogenic microbes) to various environmental compartments, where different living beings could be contacted, and eventually infected, taking into account that mutations can alter infectivity of the current strains. This could be a serious matter of concern (Carducci et al., 2020; Núñez-Delgado, 2020a), even if the fecal-oral route is not demonstrated for COVID-19, and the survival of SARS-CoV-2 in water is limited, as it could change in the coming future.

Recently, Carraturo et al. (2020) have reviewed data on the persistence of SARS-CoV-2 in the environment, being one of the very scarce papers considering sewage sludge within this matter. Also, Núñez-Delgado (2020b) and Conde-Cid et al. (2020) have published the first papers where the main focus is placed on soils as regard SARS-CoV-2.

However, up to the date, no paper has been published dealing with SARS-CoV-2 in soil-related liquid samples. In view of that, we will comment on the convenience of going ahead in the development of a methodological approach for sampling, concentration and quantification of SARS-CoV-2 (or other pathogenic microorganisms) in soil-related liquid samples.

2. Field studies regarding soil-related liquid samples

For field sampling of soil-related liquid samples we will take some of our previous field experiments as starting point. These previous researches were focused on both chemical and microbiological (fecal bacteria) contaminants, and specific sampling materials and devices were used and situated in experimental plots where

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soils received the spreading of cattle slurry. For the current situation, and eventual future investigations dealing with SARS-CoV-2 or other pathogenic microorganisms, locations were soils receive the spreading of wastewater, sewage sludge or other wastes with potential to contain and shed these microbes to soils, soil-related liquids and/or plants, could be selected to perform experimental plots.

In all cases, all devices installed in the plots, as well as all materials used for further sampling, will be sterilized prior to use, or disinfected *in situ* when needed.

In some of our previous works (for example López-Períago et al., 2002; Núñez-Delgado et al., 2002) we described different kinds of experimental plots and devices installed to allow sampling of soil-related liquids, such as lixiviates, leachates, or runoff. In Fig. 1 we include images of some of these devices, which could be used for equivalent researches focusing on SARS-CoV-2, other coronaviruses, or other pathogenic microorganisms.

All soil-related liquid samples must be kept cold to reach the laboratory in conditions allowing the survival of microorganisms.

3. Laboratory studies regarding generation of soil-related liquid samples

Soils must be sampled following standardized procedures to allow representative results, also using sterilized material. These soil samples may be taken in areas receiving the spreading of waste materials containing the microbes to be studied, or may be soil samples from not polluted zones, which could receive the

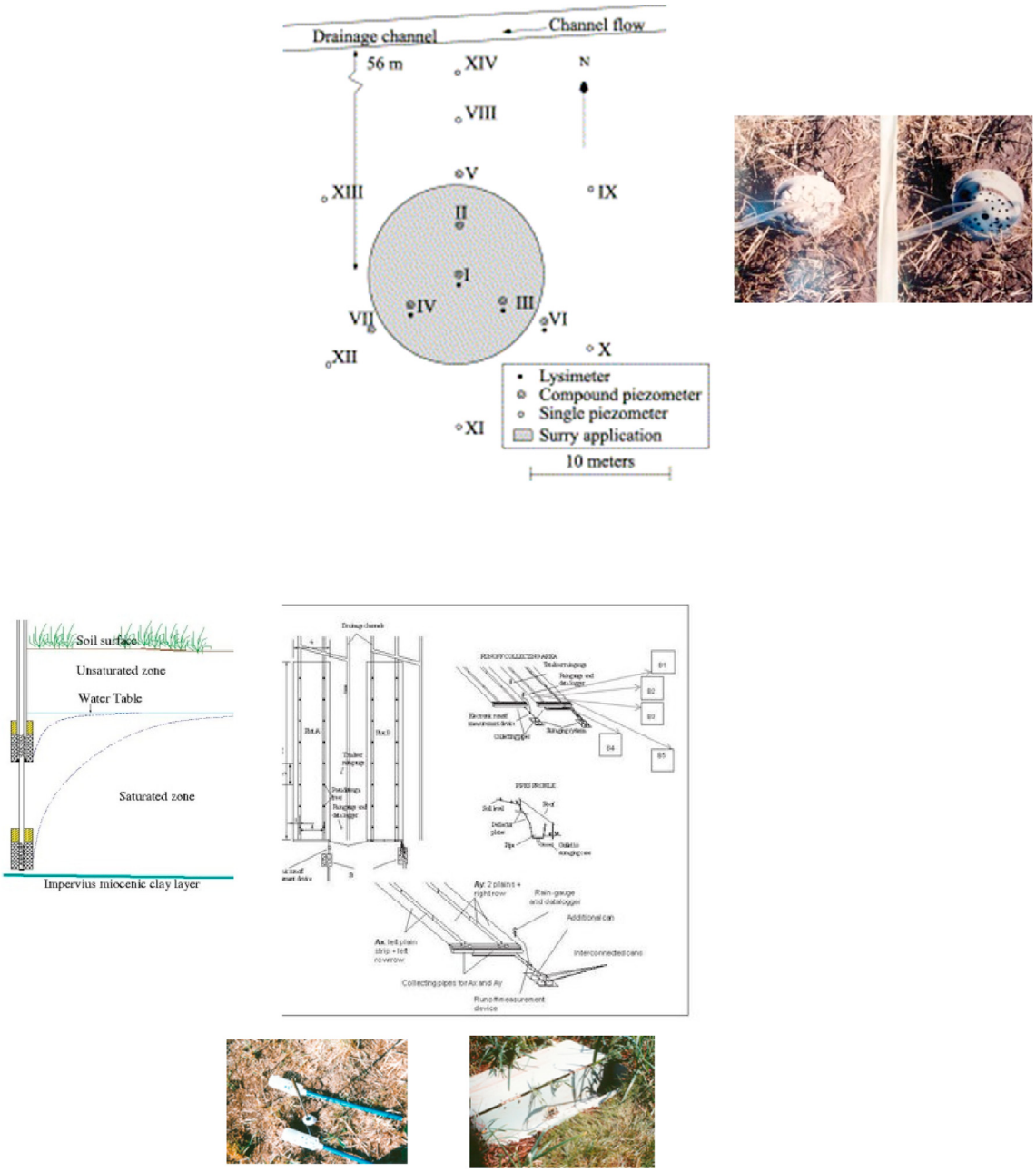

Fig. 1. Some of the layouts of experimental plots and details of some of the devices used in our previous works where soil-related liquid samples were taken for chemical and microbiological determinations.

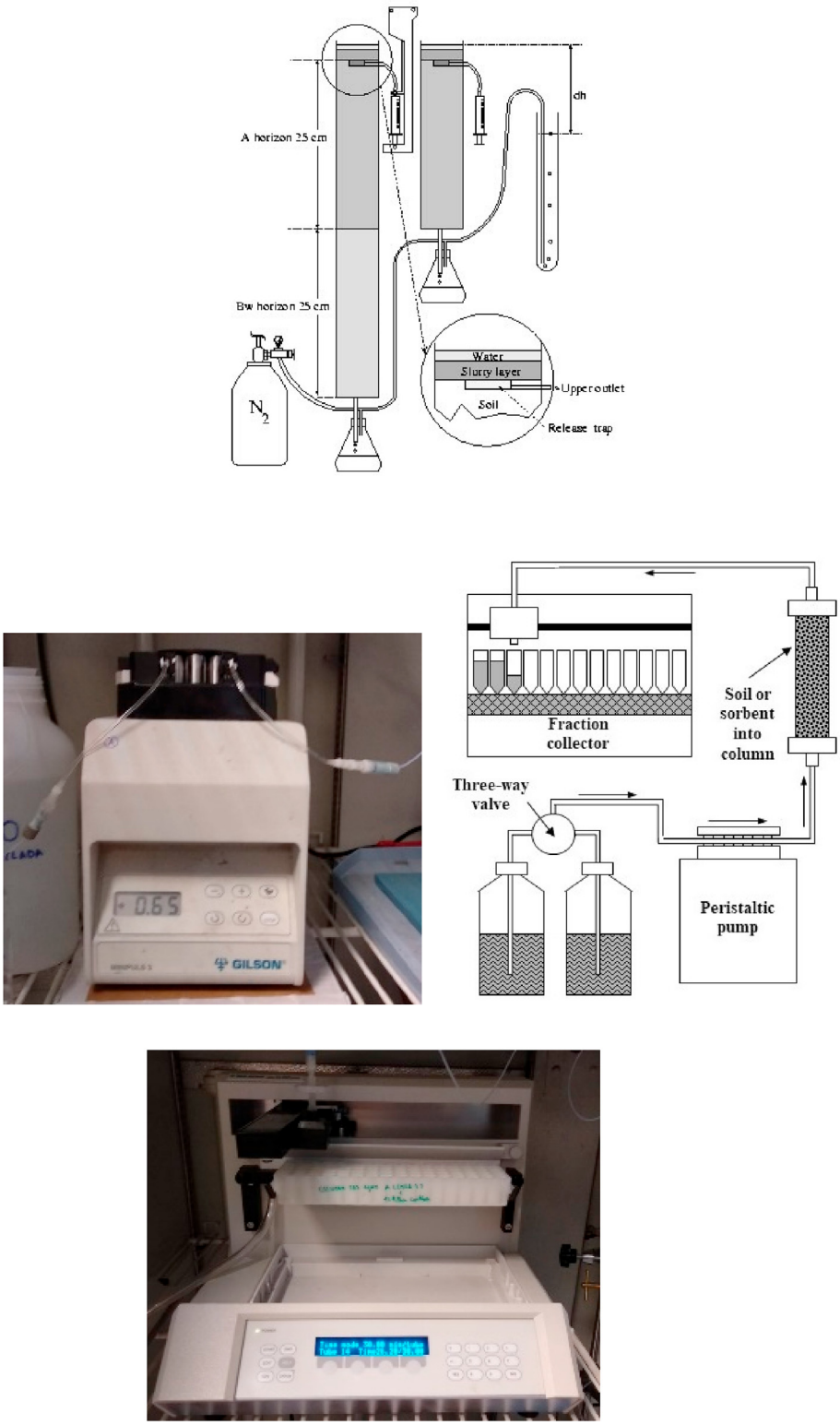

Fig. 2. Schematic details and images corresponding to various laboratory columns and accessories used in some of our previous experiments where chemical and microbiological parameters were determined.

application of the desired wastes or specific microbes at the laboratory. In all cases, soil-related liquid samples would be generated at the laboratory.

As in the case of liquid samples taken from experimental plots, all solid soil samples must be kept cold to reach the laboratory in conditions allowing the survival of microorganisms. At the laboratory, soil column experiments can be performed to generate liquid samples. Fig. 2 shows some of the columns and additional devices

used in previous laboratory experiments where we generated soil-related liquid samples to study chemical and microbiological parameters (see for example López et al., 1998; López-Periago et al., 2000; Núñez-Delgado et al., 1996). All materials must be sterilized previously, and researchers must wear the appropriate protective equipment. All samples must be processed following specific procedures to avoid contamination during collection and additional steps for quantification, as well as carry out an appropriate further sterilization/disposal of all samples and related materials.

4. Concentration and quantification of SARS-CoV-2 (or other pathogenic microbes)

Taking into account that there is no specific procedure for the determination of SARS-CoV-2 in soil-related liquid samples, the procedure described by Randazzo et al. (2020) for wastewater, regarding concentration and quantification, could be assayed as starting point, and may be it could facilitate the development of more specific methods for soil-related liquid samples.

These authors validated an aluminum hydroxide adsorption-precipitation concentration method, and then used it to monitor the occurrence of SARS-CoV-2 in wastewater samples, quantifying by means of the real-time RT-PCR (RT-qPCR) Diagnostic Panel validated by US CDC that targets three regions of the virus nucleocapsid (N) gene.

In addition, some other alternatives could be considered as starting point for developing specific methods for SARS-CoV-2 in soil-related liquid samples, as those reviewed by Farkas et al. (2020b). Other previous works could be also considered as useful references in this regard, such as those by Kimura et al. (2008), Gutiérrez and Buchy (2012), Williamson et al. (2017), and Kuzyakov and Mason-Jones (2018), or those by Bibby and Peccia (2013), Martínez-Puchol et al. (2020), and Nag et al. (2020).

5. Conclusions

- SARS-CoV-2 has not been studied in soil-related liquid samples.
- The fecal-oral route cannot be discarded for current and future strains of SARS-CoV-2 and eventual future coronaviruses or other pathogenic microorganisms with epidemic/pandemic potential.
- Specific procedures could be developed for the determination of SARS-CoV-2 and other pathogenic microorganisms in soil-related liquid samples.
- Some of the devices used in previous experiments are suggested for field and laboratory sampling of soil-related liquid samples in this regard.
- Some recently published procedures conceived for the concentration and subsequent determination of SARS-CoV-2 in wastewater are suggested to be assayed and considered as eventual starting point for the development of specific procedures for soil-related liquid samples.

Author contributions

Manuel Conde-Cid: Methodology; Writing – review & editing, Manuel Arias-Estévez: Conceptualization; Methodology; Writing – review & editing, Avelino Núñez-Delgado: Conceptualization; Methodology; Writing – original draft; Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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