SARS-Cov-2 in wastewater: a seven-month period of monitoring infection dynamic in Athens

KONTOU A.[1], GALANI A.[1], KOSTAKIS M.[1], MARKOU A.[1], LIANIDOU E.[1], and THOMAIDIS N.[1],

[1] Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimioupolis Zografou, 15771 Athens, Greece

*corresponding author:
e-mail: ntho@chem.uoa.gr

Abstract. Since December 2019, COVID-19 has spread rapidly all over the world with considerable morbidity and mortality. SARS-CoV-2 infections are accompanied by the shedding of virus in feces of both symptomatic and asymptomatic individuals, indicating that Wastewater-based Epidemiology (WBE) is an appropriate chemical tool to monitor the number of infections and disease prevalence in a community level.
In the current study, SARS-CoV-2 load was measured in influent wastewater from Athens from November 2020 until May 2021. PEG precipitation and Water DNA/RNA Magnetic Bead kit were selected as the concentration and extraction method, respectively.
The study timeline is divided in three different phases based on the levels of viral load. On 7th of November 2020, Greek government announced the second strict total lockdown after March 2020. Viral load was stable during December and the first days of January, indicating the effectiveness of lockdown and restrictions. After the announcement of the third lockdown (20th of February) the viral load reached peak levels especially during April (more than 100,000 copies/L from 17th to 20th of April). On May 14th, the end of lockdown was announced, while the number of vaccinations had been increased and the viral load was significantly decreased to approximately 37,000 copies/L.
Keywords: Wastewater-based epidemiology, SARS-CoV-2, COVID-19, infection, wastewater surveillance

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread rapidly worldwide and caused more that 3 million deaths **(Barcelo)**. The latest global numbers on the Coronavirus outbreak revealed increased infections in Europe according to the World Health Organization, WHO (https://covid19.who.int/) and affect every aspect of life. Several research groups have clinically observed SARS-CoV-2 ribonucleic acid (RNA) shed in feces of infected individuals through the sewer system in Wastewater Treatment Plants (WTPs) (**Chen**). In that framework, Wastewater-Based Epidemiology (WBE) is a promising and especially useful tool in the development of an early warning and surveillance system, which aims at tracking not only Coronavirus Disease 2019 (COVID-19) pandemic, but any future epidemic and public health problems (**Daughton**).

Numerous research groups has successfully apply WBE, in order to detect SARS-CoV-2 RNA in different wastewater samples or/and evaluate the number of infections in harmed communities (**Ahmed, Haramoto, Medema).** The results were examined and evaluated by relative authorities and provided important epidemiological information in order to adopt appropriate measures on the right time.

In Greece, the first case was recorded on 26/02/2020 and the highest numbers of confirmed cases, more than 2,000 infections, reported in March and April, according to National Public Health Organization, NPHO (https://eody.gov.gr/en/).In the present study we aim to monitor COVID-19 infection dynamic in Athens, employing WBE for an extended sampling period (November 2020 to May 2021).

2. Materials and Methods

2.1. Wastewater sampling

24-hour flow proportional influent wastewater samples were collected from the WWTP of Athens, which serves approximately the 30% of total Greek population. For every sampling day, the amount of inhabitants was real-time calculated, based on concentrations of total P, total N, BOD, COD and NH4-N (**Been, Van Nuijs**) which were provided by the WWTP of Athens. All the samples were transferred to the laboratory in a fridge box (4°C) and analyzed immediately after the arrival at the laboratory.

2.2. Sample concentration and RNA extraction

PEG (Polyethylene glycol) precipitation was the selected concentration method and the isolation of virus RNA was performed using Water DNA/RNA Magnetic Bead kit (IDEXX, United States of America) as described below.

50 mL of a wastewater sample were centrifuged at 4,750 rcf for 30 minutes at 4°C. 3.5 g Polyethylene Glycol 8000 Molecular Biology Grade and 0.8 g NaCl were added in an empty centrifuge tube and mixed with the supernatant of the wastewater sample. After complete dissolvation, the mix was again centrifuged at 10,050 rcf for 2 hours at 4°C. The supernatant was discarded. The viral pellet in the centrifuge tube was reconstituted to 500 μL nuclease-free water. The RNA was extracted immediately after concentration. 200 μL of concentrated wastewater were used for extraction according to manufacturer's instructions.

2.3. RT-qPCR

RNAs were analyzed for the detection of N1 and N2 genes of SARS-CoV-2 by RT-PCR using Water SARS-CoV-2 RT-PCR Test (IDEXX, United States of America). Reaction volume was set at 25 μL and performed in CFX96 Touch Real-Time PCR Detection System (Bio-Rad, United States)

3. Results

On 7th of November, Greek government announced the second total strict lockdown due to high confirmed COVID-19 cases. After restrictions, the viral load was stable especially during December and the first days of January, indicating the effectiveness of lockdown measures (**Figure 1**). After the announcement of the third lockdown (20th of February) the viral load reached peak levels (more than 100,000 copies/L from 17th to 20th of April). According to Figure 1, during the 4th week of April, SARS-CoV-2 reached the highest levels from the beginning of the study period. The observed increase could be explained by SARS-CoV-2 mutation during the aforementioned period. On 14th of May, the end of lockdown was announced, while the number of vaccinations had been increased and the viral load was significantly decreased to approximately 37,000 copies/L.

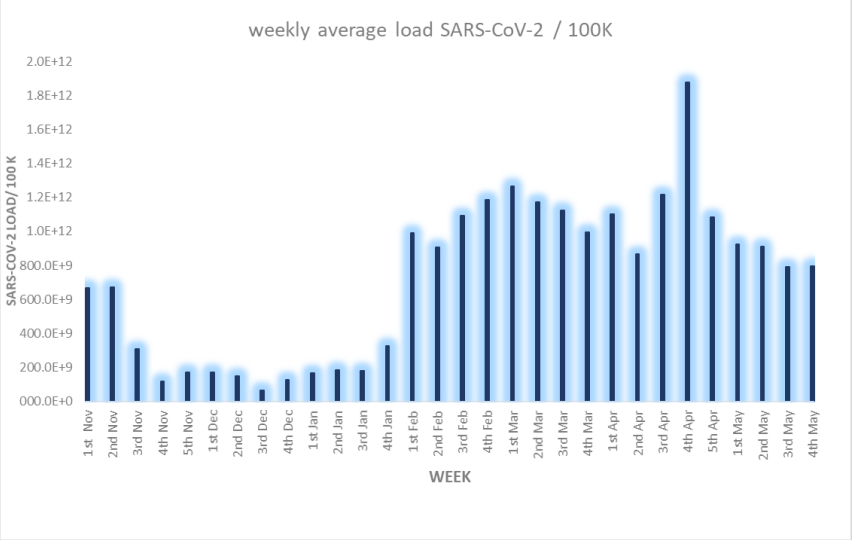

Figure 1. Weekly average of SARS-CoV-2 load per 100,000 inhabitants from November to May.

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