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Occurrence and removal of microplastics in a municipal wastewater treatment plant with conventional activated sludge process: A case study in Isfahan, Iran

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Abstract

Background: Microplastics (MPs) are nowadays found in the air and in various terrestrial and aquatic environments and have become emerging pollutants. These particles can absorb other chemicals and microbial contaminants and release them into the environment and food chain. Despite the high efficiency of wastewater treatment plants (WWTPs) in removing MPs, WWTPs are still one of the major sources of MPs discharge to the environment. This study was conducted to evaluate the efficiency of MPs removal in a municipal WWTP with conventional activated sludge in Iran.

Methods: MPs particles were counted using a stereomicroscope after the initial preparation steps (sieving, chemical digestion with the catalytic wet peroxidation-oxidation and density separation with NaCl) and then analyzed for particle composition using a Raman micro-spectrometer.

Results: MPs concentration in the influent, grit chamber, primary sedimentation tank, and effluent were 843.2 ± 147.5, 315.5 ± 54.7, 80.2 ± 19.1, and 11.13 ± 3.14 items/L, respectively. The overall MPs removal efficiency of the WWTP was 98.7%, with the grit chamber, primary sedimentation tank, and secondary sedimentation tank removed 62.6%, 27.9%, and 8.2% of the total MPs, respectively. The most abundant polymers were polypropylene (PP) and polyethylene (PE).

Conclusion: Despite the effective removal of MPs in WWTP, on average $4.47 \times 10^{11} \pm 1.03 \times 10^{11}$ MPs are discharged into the receiving waters through the effluent of this WWTP annually. This means that WWTPs can be one of the major sources of MPs in the environment and efforts should be made to increase the efficiency of WWTPs and equip them with advanced technologies.

Keywords: Microplastics, Plastics, Wastewater, Environmental pollutants, Iran

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Introduction

Nowadays, plastic materials are widely used in human activities, and the pollution caused by them has become a growing world's concern (1,2). Synthetic fibers account for more than 73% of global fiber consumption, and polystyrene (PS), polyamide (PA), polypropylene (PP), polyethylene (PE), acrylic, and polyolefin are the most commonly used plastics (3,4). Microplastics (MPs) (synthetic plastic particles < 5 mm in diameter) (4) have been found in the air and various terrestrial and aquatic environments such as oceans, rivers, lakes, urban runoff, raw and treated wastewater and composts (5-7). MPs are divided into two categories based on the origin of production; primary MPs are mainly present in small sizes such as fine particles in personal care products and cosmetics, e.g., lotions, facial and body scrubs, soaps, toothpastes, cleansers, and clothing fibers, which mainly enter the sewer system directly; and secondary MPs that result from fragmentation of large plastics (8,9). Fragmentation can occur during the use of plastics such as textiles, paints, and rubber, or after the release of plastics in nature, mainly by weathering and photolysis (5). In wastewater treatment plant's (WWTP's) effluents, secondary MPs are present in greater amounts than primary MPs (66%-88%) (10).

Due to the hydrophobic nature of MPs, they tend to absorb chemical pollutants such as polybrominated diphenyl ethers, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (4,9,11). According to reports, MPs in the marine environment

are capable of concentrating toxic chemicals up to one million times higher than their background concentration (12). Yazdani Foshtomi et al demonstrated a significant correlation between the amount of MPs and the concentration of heavy metals, TPH, and PAH in coastal sediments (13). MPs also have special physical and chemical properties such as small size, high density, color, and sometimes, a small length-to-diameter ratio, which increases the access of living organisms to this type of contaminant and naturally increases their consumption by aquatic animals. Such consumption has been observed in fish and marine species such as *Mytilus edulis, Nephrops norvegicus*, and zooplankton (1,4,9,14,15). Under such conditions, consumption of MPs by aquatic animals can cause the entry of MPs and their contaminants into the human food chain.

While the presence of MPs in the aquatic environment has been reported, the pathways of entry have not been defined yet (16); indeed, WWTPs are considered as potential sources of MPs entry into the environment (17,18). In recent years, several studies have investigated the MPs in WWTPs around the world. Murphy et al examined a secondary WWTPs in Glasgow, Scotland, for MPs and found that influent and effluent contained 15.7 ± 5.23 and 0.25 ± 0.04 items/L, respectively (19). They found that 98.41 of MPs are removed at this WWTP, but despite the high removal efficiency, an estimated 65 million MPs is discharged to the receiving water each day. Simon et al examined raw and treated wastewater from 10 of the largest WWTPs in Denmark for MPs, and found 7216 and 54 items/L, equal to 250 and 4.2 µg/L in raw and treated wastewater, respectively (20). The authors estimated that Danish WWTPs discharge about 3 tons of MPs per year. Hidayaturrahman and Lee (21) studied the removal of MPs in WWTPs with different technologies. Their study revealed that the primary and secondary WWTPs remove MPs with efficiencies ranging from 75% to 91.9%, and the removal efficiency of tertiary WWTP is over 98%.

As mentioned above, the high removal efficiency of MPs in WWTPs is confirmed by numerous studies. WWTPs can usually remove more than 90% of MPs, and the highest removal occurs after tertiary treatment (22). However, since these WWTPs are not specifically designed to remove MPs, significant amounts of MPs are released into the environment via effluent from the treatment plants (22,23). Following the WHO report, which shows the importance of more research for MPs in different stages of treatment plants (22), this study aimed to investigate the concentration of MPs in different stages of a municipal WWTP with conventional activated sludge process in Iran, and also, to determine the MPs removal efficiency in different stages and the MPs discharge rate of this WWTP.

Materials and Methods

To date, no standard method for MPs sampling and analysis has been published by any organization. However, ISO is investigating this issue and has conducted studies to compare MPs measurement methods, and extensive studies are being conducted worldwide to achieve this goal (22). The MPs measurement steps in various studies usually include sampling and sieving, pretreatment (digestion), separation based on density difference, counting, and identification of the chemical structure of MPs, which were also used in this study. Each of these steps is described below:

Sampling and sieving

In this study, a municipal WWTP with conventional activated sludge process in Iran was investigated. The WWTP treats municipal wastewater with a capacity of 110 000 m^3/day and its processes include pumping station, mechanical screening, grit chamber, primary sedimentation tank, aeration tank, secondary sedimentation tank, and chlorination tank. Sampling at this WWTP was conducted three times in September, October, and November 2020. Each time, approximately 1.5 liters of influent wastewater (S_1), 2 liters of wastewater after grit chamber (S_2), 5 liters of wastewater after primary sedimentation tank (S_3), and 50 liters of effluent (S_4) were sampled and sieved using 3 sieves with 5 mm, 300 µm, and 53 µm pore sizes. The material on the 300 and 53 µm sieves was then rinsed with distilled water into the glass containers and taken to the laboratory. The 300 µm sieve was used only to prevent clogging of the 53 µm sieve.

Digestion and density separation

Digestion was performed according to the study of Lares et al (24) and Masura et al (25) using the catalytic wet peroxidation oxidation method. In this method, hydroxyl radicals produced during the decomposition of H_2O_2, oxidize most organic materials to H_2O, CO_2, aldehydes, and carboxylic acids. In addition, $FeSO_4$ enables rapid digestion of organic materials (26). For this purpose, samples were transferred to a 1-L beaker and dried at 75°C. Then, 20 ml of $FeSO_4$. 0.05 M (Merck, Germany) and 20 mL of H_2O_2.35% (Dr. Mojallali, Iran) were added to the samples. They were then covered with a watch glass and placed on a hot plate at 75°C. After observing the reaction bubbles, they were removed from the hot plate and placed under a laminar flow hood. Then, they were placed on the hot plate for 30 minutes. Finally, the samples were cooled at room temperature (25). To prepare $FeSO_4$.0.05 M, 7.6 g of $FeSO_4$ powder was added to 1 liter of distilled water. To completely dissolve the powder, 3 ml of sulfuric acid was also added to the solution (25,27).

The samples were then passed through a fiberglass filter (Whatman, GF-3, 125 mm, 0.6 µm) (28,29) using a vacuum set, and the filters were rinsed with a NaCl-

saturated solution (Dr. Mojallali Co, Iran) (30-33) with a density of 1.2 $\frac{g}{ml}$ into a 500-mL decanter. The decanters were filled with NaCl-saturated solution and kept at room temperature for 24 hours to float the MPs, and then, the supernatant was decanted into a beaker. Then, the density separation for the sediment was repeated two more times (34). Finally, the liquid containing possible MPs was transferred to a fiberglass filter for quantitative and qualitative analysis.

Qualitative analyses

The UniRAM (South Korea) Raman spectrometer equipped with a solid-state laser with an excitation wavelength of 785 nm and a power of 200 mW was used for qualitative analyses. Two cut-outs (1 cm × 1 cm) from each filter were attached to the Au-coated glass holder and Raman spectra were recorded. Two spectra were recorded from each cut-out. The spectra were first baseline corrected using Origin 2019 software, and then, compared to the reference spectra (12) to identify the MPs type.

Quantitative analyses

The number of MPs on each filter was counted using a stereo microscope (Japan, Olympus stereo zoom microscope SZX7) with 10-80x magnification. The mean and standard deviation of 3 samples were used to describe the MPs concentration. The efficiency of MPs removal in each stage was calculated based on the difference of MPs concentration before and after stage, and the overall efficiency of the WWTP in MPs removal was analyzed based on the difference of MPs concentration in influent and effluent. In addition, the results of the quantitative analysis of MPs were modified using the results of the qualitative analysis, and 10.4% of the particles identified as unknown particles were subtracted from these results. Then, a 1 cm × 1 cm cut-outs of each filter were photographed using scanning electron microscope (SEM) (SERON TECHNOLOGY, South Korea, AIS2100).

Quality assurance and quality control

For the quality assurance and quality control of the study, the following steps were taken; each measurement was performed in triplicate. The concentration of MPs was corrected using the percentage of non-plastic materials determined by Raman micro-spectroscopy. All equipment, including sampling containers, vacuum set, beakers, Petri dishes, etc., were glass types and were first washed with acid. The work surfaces were cleaned with 70% ethanol. Air movement in the laboratory was controlled by closing the windows and door, and analyses were performed under a laminar flow hood (12,25).

Statistical analysis

The mean values and standard deviations of triplicate samples from different sampling days were calculated and expressed as MPs abundance. All statistical analyses were calculated and graphed using Microsoft Excel (version 2017).

Results

Characterization and abundance of MPs

A total of 48 spectra were obtained from the samples using Raman micro-spectroscopy and compared to the reference spectra. The Raman micro-spectroscopy analyses revealed that 89.6% of the particles were plastics. 10.4% of the particles had unknown spectra and were identified as non-plastic particles and excluded from the MPs concentration. The most common polymers were PP, PE, PA, PS, and polyvinyl chloride (PVC). The abundance of the polymers detected in the WWTP is shown in Figure 1, and the spectra of the identified polymers are also shown in Figure 2.

The concentration of MPs in the WWTP influent, grit chamber effluent, primary sedimentation tank effluent, and WWTP effluent was 843.2 ± 147.5, 315.5 ± 54.7,

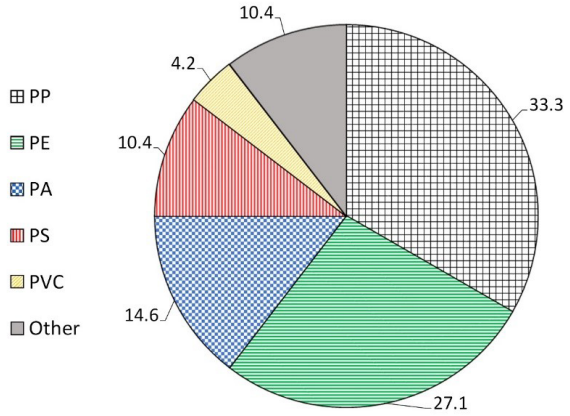

Figure 1. The abundance of polymers detected in the WWTT

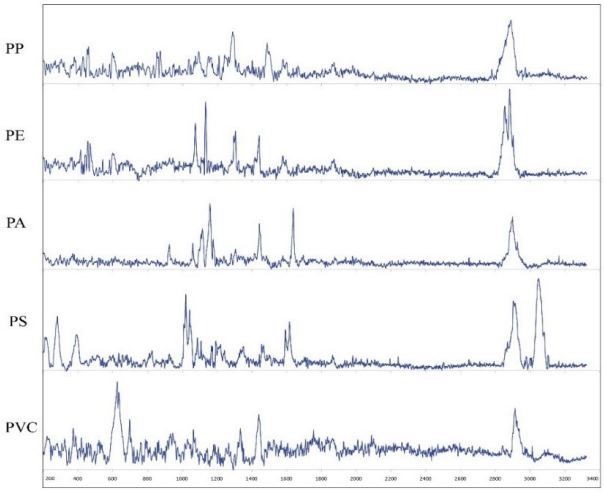

Figure 2. Representative Raman spectrum of various MPs identified in different stages of the WWTP

80.2 ± 19.1, and 11.13 ± 3.14 items/L, respectively. The abundance of MPs in different stages is shown in Table 1. The SEM and stereomicroscope image of MPs are shown in Figures 3 and 4, respectively.

MPs removal efficiency in WWTP

The MPs removal efficiency in grit chamber, primary sedimentation tank, and secondary sedimentation tank were 62.6%, 74.6%, and 85.9%, respectively. The overall MPs removal efficiency of WWTP was 98.7%, which grit chamber, primary and secondary sedimentation tank removed 62.6%, 27.9%, and 8.2% of total MPs, respectively. Despite the high efficiency of WWTPs in removing MPs, a portion of MPs is not removed (35), and even in the effluents of advanced WWTPs, some MPs remain (36), and since municipal WWTPs treat millions of liters of wastewater daily and discharge their effluent, they can be considered as important sources of MPs in the environment. In this study, it was found that despite the effective removal of MPs in a municipal WWTP (98.7%), considering that this WWTP discharges 40 MCM of treated wastewater into the receiving water (river) on average, $4.47 \times 10^{11} \pm 1.03 \times 10^{11}$ MPs are discharged into the receiving water annually through the effluent of this WWTP.

Discussion

Since there is no standard method for sampling and analyzing MPs and the measurement methods affect the amount of MPs that can be identified, it is difficult to compare the results of different studies (37). However, in recent years, several studies have confirmed the presence of MPs in WWTPs around the world. In this study, MPs were identified as in previous studies. The concentrations of MPs in the influent and effluent of various WWTPs are shown in Table 2. As shown in this table, the MPs concentration in the influent of WWTPs varies from 1 particle per liter in the study of Carr et al (9) to 31 400 particles per liter in the study of Hidayaturrahman and Lee (21). The MPs concentration in the effluent of different WWTPs has also reported differently. MPs concentration in the WWTPs effluent have been varied from 8.8×10^{-4} particles per liter in the study of Carr et al (9) to 297 particles per liter in the study of Hidayaturrahman and Lee (21). This difference may be attributed to the different methods used to measure MPs or may indicate that some

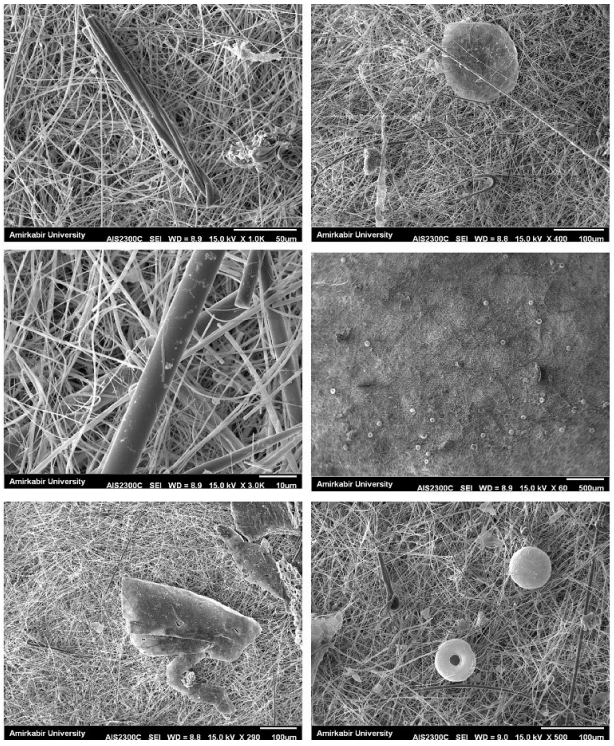

Figure 3. Typical SEM images of different MPs detected in the WWTP

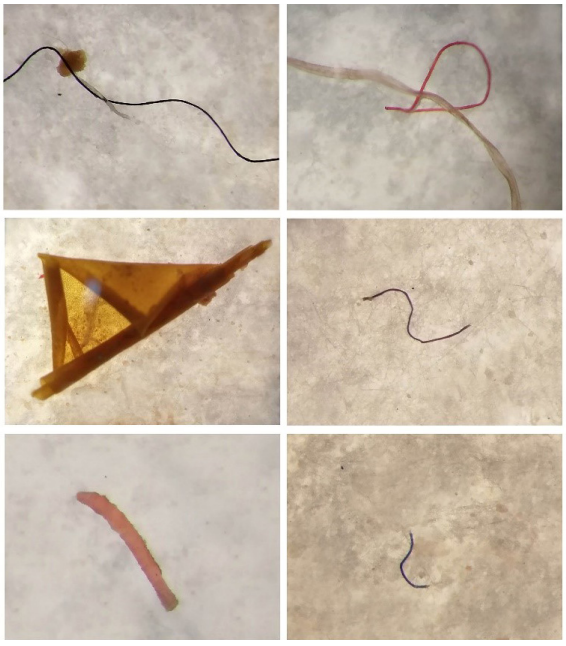

Figure 4. Typical stereomicroscope images of different MPs detected in the WWTP

Table 1. The abundance of MPs in different stages of WWTP

Sampling location	The abundance of MPs (items/L)				
	Sample 1	Sample 2	Sample 3	Mean	SD
WWTP influent	708.67	1001.00	820.00	843.22	147.54
Grit chamber effluent	292.60	378.00	276.00	315.53	54.73
Primary sedimentation tank effluent	60.57	98.80	81.20	80.19	19.14
WWTP effluent	14.31	11.04	8.04	11.13	3.14

Table 2. Abundance and measuring methods of MPs and MPs removal efficiency in WWTPs

References	Removal efficiency (%)	Effluent (items/L)	Influent (items/L)	Description	Treatment	Location	Methods			
							Separation	Finest sieve mesh or filters pore size (µm)	Detection	Digestion
Carr et al (9)	99.9	8.8×10^{-4}	1	Gravity filtration	Primary, secondary, and tertiary	USA	-	45	Visual/FTIR	USA
Murphy et al (19)	98.41	0.25±0.04	15.7±5.23	-	Primary and secondary	Scotland	-	65	Visual/FTIR	Scotland
Leslie et al (39)	72	51-81	68-910	-	Primary and secondary	Netherland	NaCl	0.7	Visual/FTIR	Netherland
Talvitie et al (8)	99.9	0.005	6.9	MBR	Primary, secondary, and tertiary	Finland	-	20	Visual/FTIR	Finland
	97	0.02	0.7	Rapid sand filtration						
	95	0.1	2.0	Dissolved air flotation						
	40-98.5	0.03-0.3	0.5-2.0	Disc filtration						
Xu et al (4)	95.1	16.3±1.2	334.1±24.3	Sand filtration and activated carbon filtration	Primary, secondary, and tertiary	China	-	5	Visual/FTIR	China
Simon et al (20)	99.3	54	7216	-	Primary and secondary	Denmark	-	10	Visual/FTIR	Denmark
Magni et al (40)	84	0.4±0.1	2.5±0.3	Sand filtration	Primary, secondary, and tertiary	Italy	NaCl	63	Visual/FTIR	Italy
Long et al (3)	79.3-97.8	0.2-1.73	1.5-13.69	-	Primary and secondary	China	NaCl	43	Visual/Raman	China
Hidayaturrahman and Lee (21)	99.2	33	4200	Ozonation	Primary, secondary, and tertiary	South Korea	-	1.2	Visual	South Korea
	99.1	297	31400	Membrane disc-filtration						
	98.9	66	5840	Rapid sand filtration						
Jiang et al (41)	75.7	30.6±7.8	126±14	-	Primary and secondary	China	NaCl & $ZnCl_2$	38	Visual/Raman	China
Edo et al (42)	93.7	12.8±6.3	183±84	-	Primary and secondary	Spain	NaCl	25	Visual/FTIR	Spain
This study	98.7	11.31±3.14	843.22±147.54	-	Primary and secondary	Iran	NaCl	53	Visual/Raman	Iran

WWTPs are using ineffective treatment processes or are not designed for sufficient MPs removal (22).

The most commonly detected polymers in this study were PP and PE. However, it should be noted that the organic and inorganic materials bound to the MPs alter the Raman spectra and these spectra do not fully match the reference spectra (38). The abundance of MPs is consistent with the results of Long et al (3). They reported that PP and PE are the most abundant MPs in WWTPs.

Most of the MPs removal (62.6%) occurs in the grit chamber, which is consistent with the results of the study of Murphy et al (19). They found that a significant portion of MPs accumulates in the grit chamber waste and in total 98.41 of MPs are removed at the WWTP. The MPs removal efficiencies in WWTPs in different studies are shown in Table 2. As shown in this table, MPs removal efficiencies in WWTPs range from 40% (8) to 99.9% (8). In general, most primary and secondary WWTPs remove more than 90% of MPs, and most MPs are removed during the pretreatment and primary treatment stages (22). Final treatment technologies such as membrane bioreactors, dissolved air flotation, and rapid sand filters remove MPs even more effectively. For example, in a Finnish WWTP, 95-99.9% of MPs was removed by these treatment technologies (8). Another study showed that 99.9% of MPs was removed in the WWTPs equipped with tertiary treatment processes using gravity bed filters (9).

An important aspect of removal of MPs in WWTPs is that MPs are usually not destroyed but transferred from one phase to another. In this case, MPs are removed from the wastewater and concentrated in the sludge, and since the sludge is used as fertilizer on the land, it is a likely pathway for contamination of the environment (22).

Conclusion

MPs concentrations in the influent, downstream of the grit chamber, primary sedimentation tank, and in the effluent were 843.2 ± 147.54, 315.5 ± 54.73, 80.2 ± 19.14, and 11.13 ± 3.14 items/L, respectively. PP, PE, PA, PS, and PVC were the most commonly found polymers. The efficiency of the grit chamber, primary sedimentation tank, and secondary sedimentation tank in removing MPs were 62.6%, 74.6%, and 85.9%, respectively. The overall efficiency of the treatment plant in MPs removal was 98.7%, which grit chamber, primary sedimentation tank, and secondary sedimentation tank removed 62.6%, 27.9%, and 8.2% of total MPs, respectively. The results of this study demonstrated that despite the effective removal of MPs in a WWTP, considering that this treatment plant discharges 40 MCM of treated wastewater into the receiving water (river), on average $4.47 \times 10^{11} \pm 1.03 \times 10^{11}$ MPs are discharged into the receiving water annually through the effluent of this WWTP. More research is needed in the future to better understand the mass balance of MPs in the WWTPs including influent, various stages, sludge, and effluent. The smaller ranges of plastics and even nanoplastics can be studied with SEM.

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Ethical issues

The dissertation related to this article has been approved ethically by Isfahan University of Medical Sciences (Ethical code: IR.MUI.RESEARCH.REC.1398.584).

Competing interests

None to be declared.

Authors' contribution

Conceptualization: Hossein Movahedian Attar.
Data curation: Hossein Movahedian Attar, and Hamze Sharifi.
Formal analysis: Hossein Movahedian Attar, and Hamze Sharifi.
Funding acquisition: Hossein Movahedian Attar.
Investigation: Hossein Movahedian Attar, Bijan Bina, and Hamze Sharifi.
Methodology: Hossein Movahedian Attar, and Hamze Sharifi.
Project administration: Hossein Movahedian Attar.
Resources: Hossein Movahedian Attar, and Hamze Sharifi.
Software: Hamze Sharifi.
Supervision: Hossein Movahedian Attar.
Validation: Hossein Movahedian Attar, and Bijan Bina.
Visualization: Hossein Movahedian Attar, and Bijan Bina.
Writing–original draft: Hossein Movahedian Attar, and Hamze Sharifi.
Writing–review & editing: Hossein Movahedian Attar, Bijan Bina, and Hamze Sharifi.

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