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Seasonal fluctuations in phthalates' contamination in pond water: A case study

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Abstract

Phthalates are endocrine disruptors, reported to cause deformities and reproductive damages in animals. Numerous studies reported the presence of phthalates in water samples of rivers, wetlands, and estuaries, while the scenario in case of ponds is different, however they are reported as an integral part of biosphere. In this study, the level of phthalates' contamination in the water samples collected from the different ponds of Amritsar district for four consecutive seasons in two years was analysed. The maximal level of phthalate contamination was found in samples collected during the monsoon season (July 2015) of first year of sampling followed by post-monsoon (October 2015) and winter season (January 2016). S8 sampling site was found to be the most phthalate contaminated site followed by S1=S11>S2=S9=S4=S5=S7>S6=S3>S10. Benzyl butyl phthalate was most abundant (found in 32% water samples) followed by di-n-butyl and dimethyl phthalate, while diallyl phthalate and diethyl phthalate were not detected. The two main drivers for these seasonal variations were observed to be temperature and precipitation. Hence, this data will be useful to explain the temporal and spatial distributions of phthalates in aquatic ecosystem, as well as to devise cost-effective ways to reduce their ecological footprints.

Keywords: Ponds, endocrine disruptor, plasticizers, priority contaminants, HPLC analysis.

Introduction

Plastic industry is significantly expanding with 368 million tons of production since 2019 (Prevarić et al., 2021). Phthalates are a well-known class of plasticizers that are produced in vast amount alongside plastic (González-Sálamo et al., 2018). They are poorly water-soluble chemicals with low volatility and have been used in numerous industries for the production of plastic products, cosmetics, medical equipment, insect repellent, propellant, etc. They are used widely due to their strength, plasticity, cost-effectiveness, and durability (Kumari and Kaur, 2021). But indiscriminate usage of phthalates has contaminated the different environmental matrices (Tao et al., 2020; Weizhen et al., 2020; Zhang et al., 2021; Suresh and Jindal, 2022). In polyvinyl chloride plastics, rubber, cellulose, and styrene manufacturing for softness and flexibility, phthalates are documented as a primary plasticizer (Cao et al,. 2016; Gao and Wen, 2016). Phthalates have the ability to leach, migrate, and evaporate into the atmosphere during the manufacturing process, usage, and dumping of plastic waste as they are not chemically bonded which causes environmental pollution. Moreover, phthalates are not easily degraded by microorganisms leading to the widespread presence in sewage sludge, landfill, sediments, and various water bodies (Qu et al., 2015). High consumption rate, continuous release into the

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environment, and resistance to microbial degradation is the main factors for their ubiquitous presence in the air, water, and food (Liu et al., 2014; Kumari and Kaur, 2020).

Humans get exposed to phthalates through different pathways like inhalation, ingestion, and direct contact through the skin which can cause developmental and reproductive toxicity. Once phthalates enter the human body, they are metabolized within hours to some days (Frederiksen et al., 2007). Exposure to these toxic substances can cause detrimental health effects including cardiovascular diseases, neuro disorders, alterations in metabolic activities, teratogenicity, etc. (Pirsaheb et al., 2022; Tran et al., 2022). Phthalates have endocrine-disrupting properties (Lyche et al., 2009) and higher concentrations can cause cancer, fetal death, injuries to the liver and kidney, deformities in the body, and reproductive damage in animals (Kay et al., 2014; Posnack, 2014; Kumari et al., 2020a). Furthermore, the United States Environmental Protection Agency (USEPA) has declared butyl benzyl phthalate (BBP), di-ethyl phthalate (DEP), dibutyl phthalate (DBP), di-(2-ethlyexyl) phthalate (DEHP), di-n-octyl phthalate and dimethyl phthalate (DMP) as priority pollutants out of 25 phthalate esters available in the market (Semenov et al., 2021; Wang et al., 2021).

Among the various aquatic ecosystems, ponds are neglected. Several reports have documented the phthalate contamination in zooplankton, wastewater, sediments, river water, wetlands, and estuaries (Li et al., 2017; Wang et al., 2017; Ramzi et al., 2018; Abtahi et al., 2019; Lee et al., 2020; Schmidt et al., 2021; Jönander et al., 2022) but few studies have been reported on phthalate contamination in ponds. Ponds are exceptional freshwater resources that exhibit self-sufficient and self-regulating ecosystems. They are a significant part of the water cycle and contribute to miscellaneous roles in the biosphere. Ponds contribute to regional biodiversity and act as steppingstones between aquatic ecosystems and landscapes (Hassal, 2014). The Ramsar convention and literature studies have defined ponds as wetlands and have also revealed the significance of ponds (Linton and Goulder, 2000). Thus, contamination of pond water by toxic chemicals is a serious ecological problem. Detailed studies on rivers and wetlands are being investigated by various researchers. However, studies on pond water bodies have been often neglected and only few studies are available in the literature mostly for physicochemical, metal, and pesticides analyses (Rehan et al., 2017; Rajput et al., 2017; Rajput et al., 2018; Kaur and Hundal, 2018; Rajput et al., 2019). Phthalates' detection in water bodies has been not reported earlier in the same geographical region but similar studies have been conducted by other researchers in the adjacent areas. For example, Ajay et al. (2021) reported the presence of phthalates in sediments samples collected from Lake Basin. Dibutyl phthalate and di(2-ethlyhexyl) phthalate were found to be ranged from 6-357 ng/g DW, which could be contributed to anthropogenic activities in the adjacent areas. Keeping in view, the research was carried out to investigate the presence of phthalic esters in pond water and its seasonal fluctuation over a period of two years using reverse-phase-ultra-high-performance liquid chromatography coupled with photodiode array detector as it has high sensitivity and selectivity for the determination of contaminants (Rajput et al., 2021).

Material and Methods

Study area

Amritsar is situated in the Majha region of Punjab which falls in the north-western part of India. It is located at 31.63° N and 74.87° E with an area of 2673 Km^2 and population density of 932. The region is characterized by semi-arid conditions. The year may be divided into four seasons: monsoon (July-September), post-monsoon (September-November), winter (December-March), and summer (April-June). Weather variations can be observed during different seasons. The heat during summer is intense with a maximum temperature of 48°C. Due to western disturbances; the region is affected by cold waves during the winter season. January is the coldest month with foggy conditions and the temperature drops down below the freezing point of water.

Sample collection

For the systematic collection of water samples, the map of the study area was prepared, and gridding was done (Figure 1). The sampling points were chosen with the help of GPS (Global positioning system). Eleven different locations were selected, and the codes were allotted to the sampling points (Table 1). Sampling was carried out from 2015-2017 in July 2015 (monsoon season), October 2015 (post-monsoon season), January 2016 (winter season), May 2016 (summer season), July 2016 (monsoon season), October 2016 (post-monsoon season), January 2017 (winter season) and May 2017 (summer season). The samples were collected in prewashed glass bottles. To prevent plastic contamination no plastic products were used during sampling and analysis. The collected samples were transferred to the laboratory immediately and stored at 4°C.

Extraction procedure

All the solvents (HPLC grade) were procured from Himedia Laboratories Private Limited (India). Dichloromethane and methanol were used as a solvent for the extraction of phthalates. An aliquot of 100 mL of each sample was taken in a separating funnel, and 10 mL of methanol and dichloromethane (1:1, v/v) was added and shaken several times till an organic layer of 2 cm was formed. All the samples were extracted thrice, the obtained extract was reduced to 1.0 mL using a rotary evaporator and then the residue was dissolved in 2 mL acetonitrile followed by filtration using a 0.22 μm membrane filter.

Table 1. Sampling sites along with their coordinates.

Sampling site	Code	Coordinates	
		Latitude	Longitude
Baserke Gallan	S1	31°61’77” N	74°71’90” E
Ajnala	S2	31°84’00” N	74°76’00” E
Raja Sansi	S3	31°72’45” N	74°78’60” E
Manawala	S4	31°74’06” N	74°68’83” E
Majitha	S5	31°76’00” N	74°95’00” E
Lopoke	S6	31°71’70” N	74°63’27” E
Attari	S7	31°69’31” N	74°65’79” E
Jandiala	S8	31°58’93” N	75°05’68” E
Sathiala	S9	31°55’50” N	75°26’55” E
Mehta	S10	31°63’39” N	74°87’22” E
Kathunangal	S11	31°73’24” N	75°02’31” E

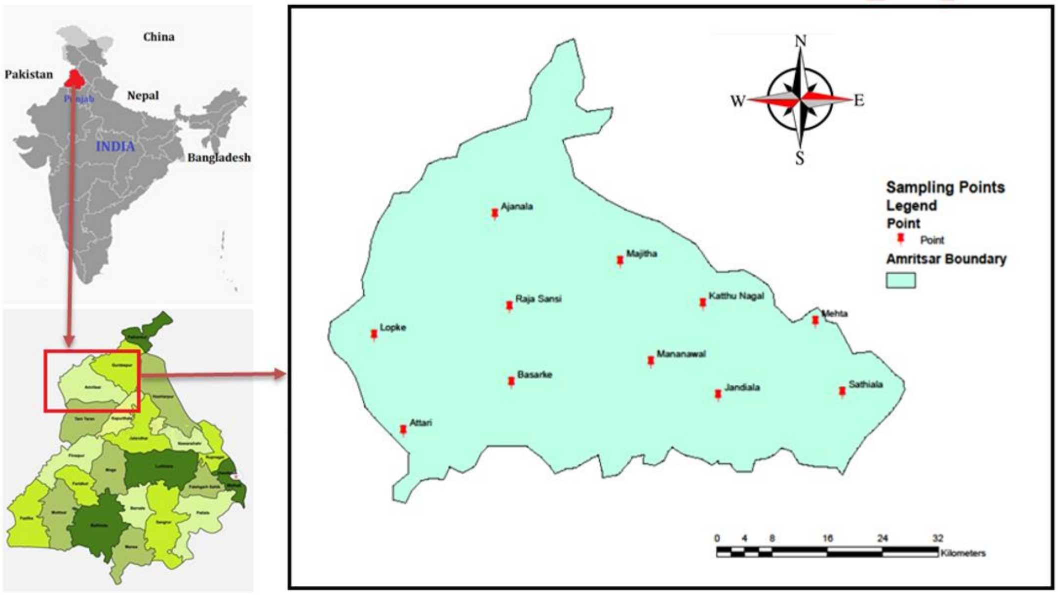

Figure 1. Map of the study area with sampling sites.

Phthalates estimation and chromatographic conditions

The quantification of phthalates (*i.e.,* dimethyl phthalate, diethyl phthalate, di-n-butyl phthalate, diallyl phthalate, benzyl butyl phthalate) in water samples was executed using a validated reverse-phase-ultra-high-performance liquid chromatography coupled with photodiode array detector (RP-UHPLC-PDA) (Kumari et al., 2020b). The stock solution (1000 mg/L) of phthalates was prepared in HPLC acetonitrile and working concentrations ranging from 2.5-1000 mg/L were prepared by serial dilution method. The operating conditions of HPLC for phthalate estimation are given in Table 2. The use of plastic or plastic products was avoided to evade the background contamination of phthalates as well as potential interference. The procedural blanks were included during the whole analytical procedure. The final concentrations of phthalates were corrected by excluding the concentrations if detected in procedural blanks (Kumari et al., 2020b). The values of limit of detection (LOD) were 0.69, 0.93, 0.68, 0.67, and 0.93 for BBP, DAP, DBP, DEP, and DMP, respectively. Correspondingly, the recorded values for limit of quantification (LOQ) were 2.08, 2.81, 2.05, 2.03, and 2.81 (Kumari et al., 2020b).

All the experiments were performed in triplicate and the results were expressed as mean ± standard error.

Table 2. Operating Conditions of HPLC for phthalate estimation (Kumari et al., 2020).

Phthalates	Dibutyl phthalate (DBP), Benzyl butyl phthalate (BBP) and Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Diallyl phthalate (DAP)
Column and column temperature	C_{18} column and 40°C
Flow rate and injection volume	0.9 ml/min and 20 μL
Detection wavelength	226 nm

Results and Discussion

Urban water bodies are more prone to contaminated with different pollutants like phthalates due to the discharge of untreated effluents from industries and municipal sewage (Zeng et al., 2009). The half-life of phthalates in the aquatic environment can range from days to several weeks depending upon the environmental conditions and chemical structure of the phthalate (Staples et al., 1997). Phthalates enter surface water through non-point sources like disposal of plastic material, road runoff, aerosol deposition, industrial discharge, agricultural activities, etc. (Roslev et al., 2007). Phthalates can occur in the aquatic environment due to the lack of covalent bonding with the polymeric matrix which accelerates their presence in the environment. This release of phthalates in the environment can occur either during the industrial processes or by leakage from the final product (Gani et al., 2017). Humans get exposed to phthalate toxicity through different pathways like inhalation, ingestion, and direct contact through the skin which can cause developmental and reproductive toxicity. In this context, Wang et al. (2014) reported daily phthalate exposure ranging from 2.6 to 7.4 µg/kg/day from indoor air and dust. Phthalate exposure leads to higher concentrations of phthalate metabolites in urine, blood, and serum of human beings. These metabolites are formed in subsequent oxidation of parent phthalates to mono phthalate ester in the human body (Singh and Li, 2012). Benzyl-n-butyl is converted into monobenzyl phthalate. Net et al. (2015) found monobutyl phthalate (71.42 µg/L) in human urine.

The present study revealed the presence of different phthalates in pond water samples (Table 3). The effect of seasonal variations on phthalate concentration was also found in the study. It was observed that maximum phthalates were detected in the water samples collected during July 2015 (monsoon season) followed by October 2015 (post-monsoon season) and January 2016 (winter season). No phthalate was detected in the samples collected during January 2017 (the winter season of the second year of sampling). The summer season of both years exhibited a lower concentration of phthalates which can be due to the higher biodegradation rate of phthalates in summers (Liu et al. 2010). Moreover, lower concentrations of phthalates in the summer season can be due to dilution, which is further affected by precipitation. Loraine and Pettigrove (2006) advocated that more use of personal care products in the summer season can cause increased levels of phthalates in surface water. Seasonal variations in the presence of endocrine disrupters in the aquatic environment have been reported in preceding studies also. In a study, Zhou et al. (2016) reported higher concentrations of steroid hormones in Lake Water in November than in May. Likewise, Kim and Kannan (2007) reported peaked concentrations of perfluorinated acids in summer season than winter season in various lakes in Albany, USA. Furthermore, Wang et al. (2012) reported a lower concentration of bisphenol-A in May than in November, whereas an inverted trend was observed in the case of 4-nonylphenol. Seasonal fluctuation in the occurrence of phthalates is important as they mainly originate from several non-point sources and their existence could be inflated by precipitation and hydrological situations which contribute to significant seasonal variations in different regions (Muller et al., 2019; Luo et al., 2021).

Among the different samples of monsoon season (July 2015) benzyl butyl phthalate was detected at eight sites *viz*., S1, S2, S4, S5, S6, S7, S9, and S11. The minimum concentration of 0.16±0.02 mg/L was found at the S9 site whereas the highest concentration of 2.43±0.01 mg/L was observed at the S2 site. Di-n-butyl phthalate was found at two sampling sites *i.e.,* S2 and S4 with concentrations of 48.66±1.29 and 39.54±1.59 mg/L respectively. Di-n-butyl is extensively used in polyvinyl chloride and polyvinyl acetates to increase flexibility. It is also used in various cosmetic products. In the natural environment, the concentration usually ranges from 30-100 µg/L whereas in urban areas it can reach from 10-1472 mg/L (Hu et al., 2020). In July 2015, water samples did not show the presence of dimethyl phthalate. During October 2015 (post-monsoon season), benzyl butyl phthalate was the only detected phthalate. Its maximum concentration was detected in the water sample collected from the S1 sampling site whereas minimum concentration was detected at the S5 sampling site *i.e.,* 2.78±0.07 mg/L and 0.29±0.01 mg/L respectively. Benzyl butyl phthalate is commonly used as an adhesive in packaging material, perfumes, vinyl gloves, etc. (Li et al., 2021). Exposure of BBP has been reported to cause eczema, rhitis, decrease in ovary weight and uterine and increase in liver size (Ema and Miyawaki, 2002; Bornehag et al., 2004). Gao et al. (2019) detected the presence of BBP in sediments and water of Taihu Lake of China with concentration of 1.30 mg/Kg and 4.72 µg/L respectively. The dissemination of phthalates in water bodies is affected by seasonal variations. In monsoon season, the high surface water runoff could act as a causative agent for higher contamination of phthalates in monsoon and post-monsoon season. During the winter season (January 2016), BBP was detected at seven sampling sites viz., S1, S2, S3, S6, S8, S9, S11 whereas dimethyl phthalate was present only at S3 sampling site with the concentration of 1.6±0.03 mg/L. Di-n-butyl phthalate was not found in any water sample. In April 2016 sampling (summer season), only DBP was detected in the water sample collected from the S8 sampling site *i.e.* 11.61±0.59 mg/L.

Di-n-butyl phthalate is used in many personal care products like body lotions, perfumes etc. as a solvent (Ahuactzin-Pérez et al., 2018b). It is one of the chief phthalates that are present in various environmental matrices like water and sediments (Gao and Wen, 2016). DBP can enter the food chain by getting absorbed by plants and other living organisms (Muneer et al., 2021).

Table 3. Phthalate esters (mg/L) in water samples collected from different sites from July 2015-May 2017.

Phthalic ester	Site code	First year of sampling (July 2015-May 2016)				Second-year of sampling (July 2016-May 2017)			
		Monsoon	Post-monsoon	Winter	Summer	Monsoon	Post-monsoon	Winter	Summer
Benzyl butyl phthalate	S1	0.73±0.01	2.78±0.07	0.58±0.01	-	-	-	-	-
	S2	2.43±0.01	0.50±0.09	0.76±0.03	-	-	-	-	-
	S3	-	-	0.70±0.03	-	-	-	-	-
	S4	1.59±0.01	1.24±0.02	-	-	-	-	-	-
	S5	1.09±0.03	0.29±0.01	-	-	-	-	-	0.18±0.01
	S6	0.35±0.04	-	0.32±0.06	-	-	-	-	-
	S7	0.43±0.04	0.84±0.02	-	-	-	0.46±0.01	-	-
	S8	-	0.59±0.01	1.68±0.07	11.61±0.59	-	-	-	0.37±0.05
	S9	0.16±0.02	0.60±0.03	1.33±0.04	-	-	-	-	-
	S11	0.74±0.04	1.77±0.13	0.41±0.005	-	63±0.98	-	-	-
Di-n-butyl phthalate	S1	-	-	-	-	-	33.14±0.58	-	-
	S2	48.66±1.29	-	-	-	-	-	-	-
	S4	39.54±1.59	-	-	-	-	-	-	-
Dimethyl phthalate	S3	-	-	1.60±0.03	-	-	-	-	-

Results are presented as Mean±S.E.

In most of the sampling sites, where phthalates were detected, the stormwater and municipal sewage were released into the ponds. The untreated municipal sewage containing various anthropogenic contaminants such as phthalates found their way into the aquatic ecosystem. Rainfall provides a basic force for surface runoff and atmospheric deposition of contaminants in the water body as evident from the higher concentrations of phthalates in monsoon season. There was also a substantial difference in the phthalate concentration in water at different sites. Higher concentration was measured at sites which were surrounded by houses, and streets, thus receiving a great number of contaminants from surroundings. Whereas, no phthalate was detected at S10 sampling site which was well maintained pond. The results are suggestive that phthalates contamination is mainly dominated by local inputs. Furthermore, phthalates are also used as solvents for many pesticides besides getting leached from packaging material (Wang et al., 2013; Li et al., 2016). Therefore, application of pesticides and fertilizers in nearby agricultural fields could be the main reason of the accumulation of phthalates in water (Liu et al., 2010; Zeng et al., 2013).

In July 2016 (monsoon season of the second year of sampling), benzyl butyl phthalate was the only detected phthalate with a concentration of 63±0.98 mg/L at the S11 sampling site. In October 2016 (post-monsoon season), BBP (0.46±0.01 mg/L) and DBP (33.14±0.58 mg/L) was detected in water samples collected from S7 and S1 sampling sites respectively. Phthalates were not detected during the winter season (January 2017) whereas in summer season (May 2017) benzyl butyl phthalate was detected at S5 (0.18±0.01 mg/L) and S8 (0.37±0.05 mg/L) sampling sites. Benzyl butyl phthalate softens the resins without chemically binding with them thus also known as an external plasticizer (Kumari and Kaur, 2019). Due to this, BBP migrates slowly from the discarded plastic material and get diffused into an aquatic environment where it can last for long periods (Dominguez-Morueco et al,. 2014; Liu et al., 2015). When aquatic organisms are exposed to phthalates, mortality occurs due to sub-lethal effects of peroxisome proliferator activator receptors. Moreover, phthalates are known for their capacity to disrupt reactive oxygen species, plummeting cells' capability to combat oxidative stress and inducing apoptosis. These events may lead to mortality of aquatic organisms (Mathieu-Denoncourt et al., 2015). Benzyl butyl phthalate can also accumulate in tissues of organisms thus entering into the food chain by process of bioaccumulation. When these aquatic organisms are consumed by another organism of the higher trophic level, they get biomagnified in the food chain. Thus, the risk of phthalates exposure to humans increases because humans are at the top of the food chain (Chatterjee and Karlovsky, 2010; NTP-CERHR, 2003). Phthalate exposure during pregnancy can lead to various adverse health effects on mother as well as on child (Sol et al., 2020; Van den Dries et al., 2020; Philips et al., 2020; Deierlein et al., 2022). Phthalates are also reported to cross the placental barrier and may lead to suboptimal foetal development and adversarial genetic effects (Santos et al., 2021).

Di-n-butyl phthalate was the second most detected phthalates in the study. The frequent occurrence of DBP in the environment has gained a lot of attention due to its endocrine disrupting properties in animals and adversarial effects on the reproductive system in humans (Xu et al., 2014; Ahuactzin-Pérez et al., 2018a). Di-n-butyl phthalate is not soluble in water and does not degrade easily, therefore, the most found phthalate in various water bodies. Di-n-butyl phthalate can enter into waterbody through direct discharge into the aquatic environment from many plastic products as they are not chemically bonded to plastic (Liu et al., 2014). Experimental studies have proved DBP as a reproductive toxicant and anti-androgenic (Motohashi et al., 2016). Studies have been reported that DBP exposure leads to dysfunction of serum reproductive hormones (Mendiola et al., 2012; Joensen et al., 2012; Meeker and Ferguson, 2014). Dimethyl phthalate was found only in one sample during the winter season of the first year of sampling. Dimethyl phthalate easily leached into the environment from plastics tubing, plates, paper, and containers (Xu et al., 2009). Due to its wide applications in various fields, DMP has contaminated soil, air, groundwater, sediments, aerosol particles and surface water (Montuori et al., 2008; Wang et al., 2008; Wu et al., 2011; Huang et al., 2015). DMP is a stable phthalate as compared to others and has the half-life of approximately 20 years (Staples et al., 1997). Among various aquatic organism, amphipods are the most sensitive species towards DMP toxicity with LC_{50} ranges from 0.0282 to 0.377 ml/L (Mathieu-Denoncourt et al., 2016). Dimethyl phthalate can cause excess accumulation of reactive oxygen species in algal cells which can lead damage in thylakoid membranes, disruption in chloroplast structure and alterations in photosynthetic functions of cells (Gao et al., 2021). Overall, it can be concluded that the seasonal variations of phthalates in water varied with the sampling site, the surrounding aquatic environment and local atmospheric deposition. The highest number of phthalates were detected at S8 sampling site followed by S1=S11>S2=S9=S4=S5=S7>S6=S3>S10. In case of phthalates, BBP was the most abundantly found phthalate followed by DBP and DMP. Diallyl phthalate and diethyl phthalate were not detected in any water sample. The outcomes of the present research strongly indicate that the phthalate contamination in pond water is significantly associated with anthropogenic activities.

Conclusion

The current study implies the first set of data on the occurrence of phthalates in the pond water of Amritsar district (Punjab, India). The outcomes uncover the presence of phthalic acid esters in the pond water. Dumping of municipal waste containing plastic product could be the main responsible cue of phthalates' contamination. Among all phthalates, BBP was the predominant one. Furthermore, the study revealed that the climate variations have played an imperative role in phthalates' distribution as their higher concentrations were reported during the monsoon and post monsoon seasons. Thus, prevalence of phthalates in the pond water can severely induce detrimental impacts on the surrounding biota.

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