



Identification and Characterization of Microplastics on the Surface Water in Laguna de Bay, Philippines

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ABSTRACT

Laguna de Bay is the largest lake in the Philippines. It is surrounded by developing cities that pollute the lake with plastics from different industrial and domestic activities. In the study, microplastics were collected from the lake's surface water through three (3) collection points within the lake. The collection of microplastics was conducted from August 2018 to October 2018. About eight-hundred ninety (890) microplastics were collected and cataloged. Among the collection sites, 'Brgy. Sampiruhan' has the most microplastics, with a median of 15 ranging from 11-24 microplastics per 1000 L of lake water. On the other hand, 'Brgy. Napindan' has a median of 4 which ranges from 2-6 microplastics per 1000 L, and 'Brgy. San Isidro' has a median of 6 which ranges from 4-24 microplastics per 1000 L. Image analysis revealed that microplastics from this site were larger and angular. The color analysis shows signs of whitening and yellowing of the plastic materials, which suggests that the microplastics undergo photodegradation. Fourier-transform infrared spectroscopy (FTIR) found that most of the microplastics in the lake are made of polyethylene and its derivatives. Microplastics in Laguna de Bay show the continuous plastic pollution in the Philippines' largest lake.

INTRODUCTION

Microplastics are plastic materials smaller than 5 mm (Arthur et al. 2009, Bowmer & Kershaw 2010). Microplastics could be produced deliberately from plastic products such as plastic pellets, scrubbers, and microbeads (Browne et al. 2010, Arthur et al. 2009, Reddy et al. 2006), hence called primary microplastics. Secondary microplastics, on the other hand, are formed from large plastic debris through mechanical weathering, photo-oxidation, thermo-oxidation, and biological degradation (Thompson et al. 2004, Browne et al. 2007, Andrady 2011). Mechanical degradation happens when shearing forces are applied to the plastic material. This could form macro-radicals that react to oxygen-producing peroxy-radicals that initiate the degradation of the polymer chains in plastic materials (Potts 1991). The primary and secondary microplastics will roam in different bodies of water and continuously break into smaller fragments until they are invisible to the naked eye.

Microplastics are now present in oceans and shorelines worldwide (Barnes et al. 2009, Browne et al. 2011, Nelms et al. 2016). A wide range of organisms could ingest microplastics because of their size. Plastic products also

contain plasticizers and additives, which may leak into the water as the plastic material degrades. Plastic can also carry toxicants on its surface, causing more detrimental effects on marine life (Koelmans et al. 2016). Furthermore, the absorption of ultraviolet from sunlight induces photolytic, photooxidative, and thermo-oxidative reactions leading to the lysis of the polymer chains hence photodegradation (Ayako & Hirose 1999, Scott 2000, Valko et al. 2001). The absorption of ultraviolet light is facilitated by the light-absorbing functional groups (Chromophores) present in plastic polymers (Schnabel 1981). Photooxidative degradation of plastics polymers includes chain scission, crosslinking, and secondary oxidative reactions requiring the formation of free radicals (Ranby & Rabek 1981, Carlsson et al. 1976, Mc Kellar & Allen 1979, Ranby & Lucki 1980). Absorption of ultraviolet light (UV) at less than 290 nm induces the photo-Fries reaction of the chromophores of the plastic materials resulting in the yellowing of the microplastics or discoloration. UV at 330 nm can induce bleaching of the pigments added to the plastic products, which results in the fading of colors (Humphrey et al. 1973, Andrady et al. 1991). Pigments and stabilizers (i.e., chalk) protrude from the fractured polymer matrices producing

a scaly appearance and whitening the plastic surface. The prolonged mechanical stresses and photodegradation weather plastic and its byproducts (Yousif & Haddad 2013).

Laguna de Bay is the largest lake on the island of Luzon in the Philippines. The lake has an area of about 911 km² and a shoreline of 220 km. It has a shallow freshwater body. The lake is surrounded by urbanizing regions such as the National Capital Region (NCR), the Province of Rizal, and the Province of Laguna (LLDA 2015). Laguna de Bay has 21 tributaries, with the Marikina and Pagsanjan rivers as its main water source accounting for 80% of its water volume (Delos Reyes 1995). Laguna de Bay is the catch basin of waters from Metro Manila and nearby provinces. One of the rivers dumping waters in Laguna de Bay is the Pasig River (Gonzales 1987).

Furthermore, household and industrial waste from nearby provinces contributes to river pollution (Santos-Borja & Nepomuceno 2006). Plastics are included in the waste dumped in Laguna de Bay, which increases as urbanization and industrialization occur in Metro Manila and its nearby provinces (Regmi 2017). More so, the local governments surrounding the Laguna de Bay and the Laguna Lake Development Authority (LLDA) have implemented a policy to ban non-biodegradable plastic bags and promote biodegradable bags such as paper bags and eco-bags (LLDA 2011). The Philippines has a worsening problem with plastic pollution. In a survey in 2010, around 280-750 thousand metric tons of plastic debris in the ocean were traced from the country, making the Philippines one of the largest contributors of plastics in the ocean (Jambeck et al. 2015, Lebreton et al. 2017, Lebreton & Andrady 2019). Laguna

de Bay is connected to the Pacific Ocean through Manila Bay and the Pasig River in Metro Manila. In recent studies, microplastics were found and isolated in the Pasig River (Deocarlis et al. 2019).

Furthermore, microplastics were also found in mussels harvested from Bacoor Bay (Argamino et al. 2016), an inlet southeast of Manila Bay. In this study, the occurrence and characteristics of microplastics collected in the south, west, and central parts of the Laguna de Bay were proven and defined. The collection was conducted in August and October of 2018. The main objective of this study is to show the abundance and identity of microplastic pollution in Laguna de Bay.

MATERIALS AND METHODS

Sample Collection and Processing

Three (3) sampling sites (Fig. 1) were assigned in this study, namely: (a) Brgy. Napindan, Taguig City (1426.87°30'' N 12120.61°05'' E); (b) Brgy. Sampiruhan, Calamba, Laguna (1430.92°12'' N, 12153.35°10'' E) and (c) P. Burgos St., Brgy. San Isidro, Tanay, Rizal (1435.46°29'' N, 12133.76°16'' E). The sampling site in Taguig city is in the western region of Laguna de Bay. In contrast, the sampling site in Calamba city is found in the southern region of Laguna de Bay, and the sampling site in the municipality of Tanay is found in the central region of the lake. Water samples were collected 5 to 10 m away from the shoreline. Three (3) sampling sites were selected because these are the sites close to urbanized cities around Laguna de Bay. Visits to the sampling sites were done in August and October 2018



Fig. 1: Laguna de Bay and the sampling sites. N – Brgy. Napindan, Taguig, Metro Manila; I – Brgy. San Isidro, Tanay, Rizal; S – Brgy. Sampiruhan, Calamba, Laguna.

because around this time is the wet season in the Philippines, where the inflow and outflow of water in the lake is more frequent, and the samples were collected from the surface water of the Laguna de Bay in triplicates.

The surface water was manually scooped using tared buckets, and the collected water was allowed to pass through a sieve stack setup consisting of 4,700 μm (4.7 mm) and 355 μm (0.35 mm) mesh sieves. Using this sieve stack, solid materials with sizes 0.35 to 4.7 mm will be trapped in between the stack. Around 500 and 2,000 liters of water were filtered during the first and second visits. Solids trapped in the 0.35 mm sieve mesh were washed with distilled water and transferred into glass containers. The collected solids were dried at 80-100°C overnight.

The dried materials were added to 20 mL of 2 M NaOH and heated around 100°C following the alkali digestion method described by Cole et al. (2014, 2015). Undigested solids were washed with distilled water, dried, and suspended in a saturated salt solution (density = 1.2 $\text{g}\cdot\text{mL}^{-1}$). Floating solids were manually picked, washed briefly in distilled water, and examined under the stereomicroscope. Photographs were taken to catalog each microplastic fragment collected. Alkali digestion was proffered over the wet-peroxide oxidation (WPO) method due to the availability of reagents and the simplicity of the procedure of the former. However, this digestion method could not isolate polyesters and nylon materials. Alkali digestion was conducted overnight for approximately 12 h.

Density Flootation Test

The collected microplastics were segregated based on their density. The microplastics were allowed to float on distilled water (1.00 $\text{g}\cdot\text{cm}^{-3}$), 50% isopropyl alcohol (0.95 $\text{g}\cdot\text{cm}^{-3}$), and vegetable oil (0.93 $\text{g}\cdot\text{cm}^{-3}$). A drop of dishwashing liquid was added to the liquid to lessen the surface tension of the microplastics on the liquid. The salt that was used for the technique was sodium chloride (NaCl) and was left overnight.

Microscopic Examination and Image Analysis

Photographs were taken for each fragment with illumination on top of the plastic fragments using a stereomicroscope with up to 40 \times Magnification. The length of each fragment was measured from the photomicrographs using ImageJ software version 1.8.0 (Schneider et al. 2012). The roundness scores of each microplastic fragment were calculated from the circularity and aspect ratios obtained from ImageJ using the formula of Takashimizu & Liyoshi (2016). The roundness score ranges from 0 (angular) to 1 (perfect circle). The color of each microplastic fragment was obtained by image

analysis. The color of the microplastics was expressed in CIE $L^* a^* b^*$ color space. And color values were obtained using the 'color distance' package in R (Weller 2019).

Identification of Microplastics Using ATR-FTIR

Attenuated Total Reflectance - Fourier-Transform Infrared Spectroscopy (ATR-FTIR) was conducted to identify the microplastics using Spectrum Two N FT-NIR Spectrometer (Perkin-Elmer, MA, USA). Thirty-two (32) representative samples were analyzed using ATR-FTIR. The FTIR spectra obtained from the samples were matched to the FTIR spectral libraries of known polymers using the SearchIt™ data toolbox of the KnowItAll® application (Biorad Informatics Division, PA, USA).

Data Analysis and Statistical Tests

Based on the Shapiro-Wilk for normality, occurrence, length, and roundness, the microplastics' CIELAB values L^* , a^* , and b^* were not normally distributed. Hence non-parametric tests such as the Kruskal-Wallis test was employed to test the effects of the location and the collection visits. The Wilcoxon Signed Test was used to determine the significant difference between the groups. All the measurements were expressed in the median and interquartile range (IQR). A Chi-square test was done to determine the dependence of the density of the microplastics distribution to the collections site. All statistical analyses were done in R 3.6.0 (R Core Team 2020).

RESULTS AND DISCUSSION

Occurrence of Microplastics in Laguna de Bay

Nine hundred twenty-seven (927) plastic fragments were isolated from the three (3) sampling sites (Table 1) collected in August 2018 and October 2018.

After conducting the density separation, microscopic examination, and image analysis, only 890 were microplastics, and the other 37 plastic fragments were too large to be classified as microplastics. Among the three (3) sampling sites, Brgy. Sampiruhan in Calamba, Laguna, has the highest occurrence of microplastics, having a median of 15 (IQR 11-21) fragments per 1000 L of water. Sampiruhan can be described as an urban and agricultural development zone by the municipality of Calamba, furthermore during the visits. Visible plastic waste is floating around the shore. On another site, Brgy. Napindan showed fewer microplastics than Sampiruhan due to the Pasig River that allows the dumping and draining of water in the Laguna de Bay, which allows the microplastic to be flushed in and out of the lake hence the minimal concentration of microplastics in Napindan.

Table 1: Characteristics of microplastics isolated in the three regions of Laguna de Bay.

Site	Brgy. Napindan	Brgy. San Isidro	Brgy. Sampiruhan
City/Municipality and Province	Taguig City, Metro Manila	Tanay, Rizal	Calamba, Laguna
Laguna Lake Region	West	Central	South
Median (IQR)			
Occurrence of Microplastics [number of microplastics per 1000 L]	4 (2-6) b	6 (4-24) ab	15 (11-24) a
Length [micrometers]	1,803 (1,306-3,521) ^a	1,676 (1,024-2,480) ^a	1,392 (983-1,862) ^b
Roundness	0.62 (0.37 - 0.74) ^a	0.54 (0.37-0.69) ^a	0.46 (0.32-0.63) ^b
CIELAB Luminance (L*)	66.8 (59.3-85.6) ^a	42.9 (38.9-65.1) ^b	47.5 (39.9-70.6) ^b
CIELAB blue-yellow (b*)	2.6 (-4.3-20.4) ^b	14.0 (6.2-23.7) ^a	17.2 (6.0-24.8) ^a

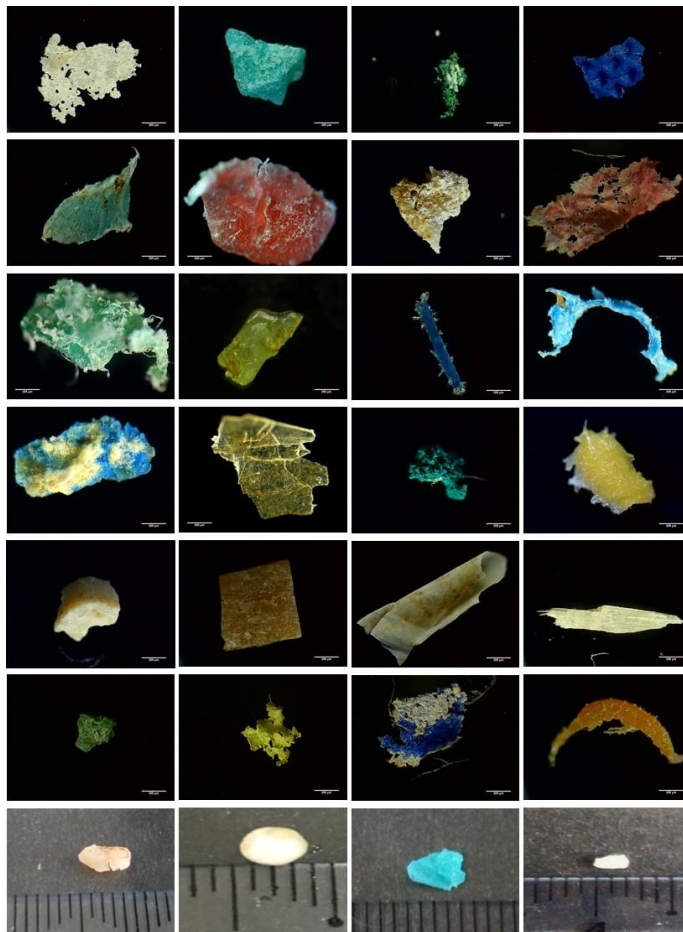


Fig. 2: Photographs of microplastics isolated from Laguna de Bay. Scale bars in the 1st to 6th rows are 355 micrometers. The graduations in the last row are 1 mm.

The occurrence of microplastics in the Pasig River (Deocaris et al. 2019) may also contribute to the concentration of microplastics in the Laguna de Bay. Napindan is an urbanized area with a thriving fishing community.

The occurrences of microplastics reported in this study were higher than in similar freshwater lakes in the

study by Dusaucy et al. (2021), wherein the occurrence of microplastics was studied in different lakes, the median microplastic occurrence is 1,442 microplastics per Liter ranging from 0.27 to 34,000 microplastics per Liter. The microplastic occurrences reported in this study are larger than the values obtained by Dusaucy in his microplastic studies.

Mechanical Weathering of Microplastics in Laguna de Bay

The length and roundness of microplastics in Laguna de Bay (see Table 1) appears to be different on each sampling site ($p < 0.05$). Microplastics from Sampiruhan were smaller and more angular compared to those collected from Napindan and San Isidro. The weathering effects that naturally happen on rocks and stones in a river also take place in large plastic materials, which results in small and round plastics until their sizes become microscopic; more so, microplastic may also collide with other materials that may contribute to the sheering forces that break the plastics more, this is comparable to small rocks in a river (Domokos et al. 2014). The same phenomenon was observed in the study conducted by Mearu (2020), wherein the polystyrene was exposed to agitating water, resulting in small and round plastic polystyrene fragments. In contrast with the angular plastics in Sampiruhan, the Napindan and San Isidro microplastics were round. This could be due to the rivers found in those sampling sites, wherein the Pasig River passes through Napindan. The Tanay River passes through San Isidro. Aside from the sheering forces that the rivers could contribute to breaking plastic materials into microplastics, the current within the different regions of Laguna de Bay may have contributed to the breaking of plastic fragments. The water at the center region of the Laguna de Bay flows southern, which rapidly converges with the water coming from the western region of the lake that flows eastern (Herrera et al. 2015). With continuous exposure to mechanical sheering forces that induce weathering, all microplastics will eventually become rounder over time; hence the angular microplastics in Sampiruhan will soon become round (Kowalski et al. 2016). More so, the rounding of microplastics will also result in loss of mass and, subsequently, the reduction of the particle density.

Photodegradation of Microplastics in Laguna de Bay

Apart from the Mechanical sheering forces from the rivers and the current within the Laguna de Bay, another factor that change the plastic material is photodegradation. Microplastics on the surface water are exposed to sunlight, an agent for photodegradation. The microplastics isolated

from Sampiruhan and San Isidro had more positive CIELAB b^* values, indicating the microplastics' yellowing due to photo-oxidation. The sunlight and heat induce the formation of free radicals due to the breakage of chemical bonds in the polymer structure, which alters the chromophores within the microplastic or polymer structure; hence the plastic changes its color (Andrady 2011, Barnes et al. 2009, Moore 2008). On the other hand, the microplastics collected from Napindan have the highest median CIELAB L^* values, which is the value for luminance. This suggests that the microplastics are whitening or turning to the color white, also an indication of discoloration, mainly through chalking, which is the formation of fine powders on the surface of the plastic or paint film due to weathering. Chalking occurs as increased amounts of polymer binder are removed from the surface of the microplastics causing pigments to protrude through the surface and produce a white and chalky appearance (McKeen 2019). More so, as the microplastics are exposed to photo-oxidation, the coloration of the microplastics turns more yellow.

Chemical Composition of Microplastics in Laguna de Bay

The microplastics isolated in Laguna de Bay are grouped into three density levels, as shown in Table 2. The distribution of microplastics within these density levels differs significantly by location ($p < 0.05$).

Around 40% of the microplastics collected in Sampiruhan have densities above the density of water (1.00 g.mL^{-1}), and plastic materials such as polystyrene, nylon, and polyethylene terephthalate are included in this density level (Kolb & Kolb 1991). Furthermore, microplastics with densities lower than 0.93 g.mL^{-1} are found in all three (3) sampling sites. Among the sampling sites, Sampiruhan has the highest number of microplastics with a density lower than 0.93 g.mL^{-1} . Several fish pens were observed in Sampiruhan, and these are commonly made of polypropylene and low-density polyethylene, and these plastics have a lower density than the water. Furthermore, these plastic polymers are less dense than water, are easily carried by currents, can stay afloat on the surface of the water, and are often exposed to sunlight, making way for more mechanical weather and photodegradation.

Table 2: Density profiles of the microplastics isolated in the three regions of Laguna de Bay.

Site	Density		
	$> 1.00 \text{ g.mL}^{-1}$	$0.93 - 1.00 \text{ g.mL}^{-1}$	$< 0.93 \text{ g.mL}^{-1}$
Brgy. Napindan	20 (19%)	66 (64%)	17 (17%)
Brgy. San Isidro	41 (22%)	124 (67%)	20 (11%)
Brgy. Sampiruhan	253 (40%)	323 (50%)	63 (10%)
Total	314 (34%)	513 (55%)	100 (11%)

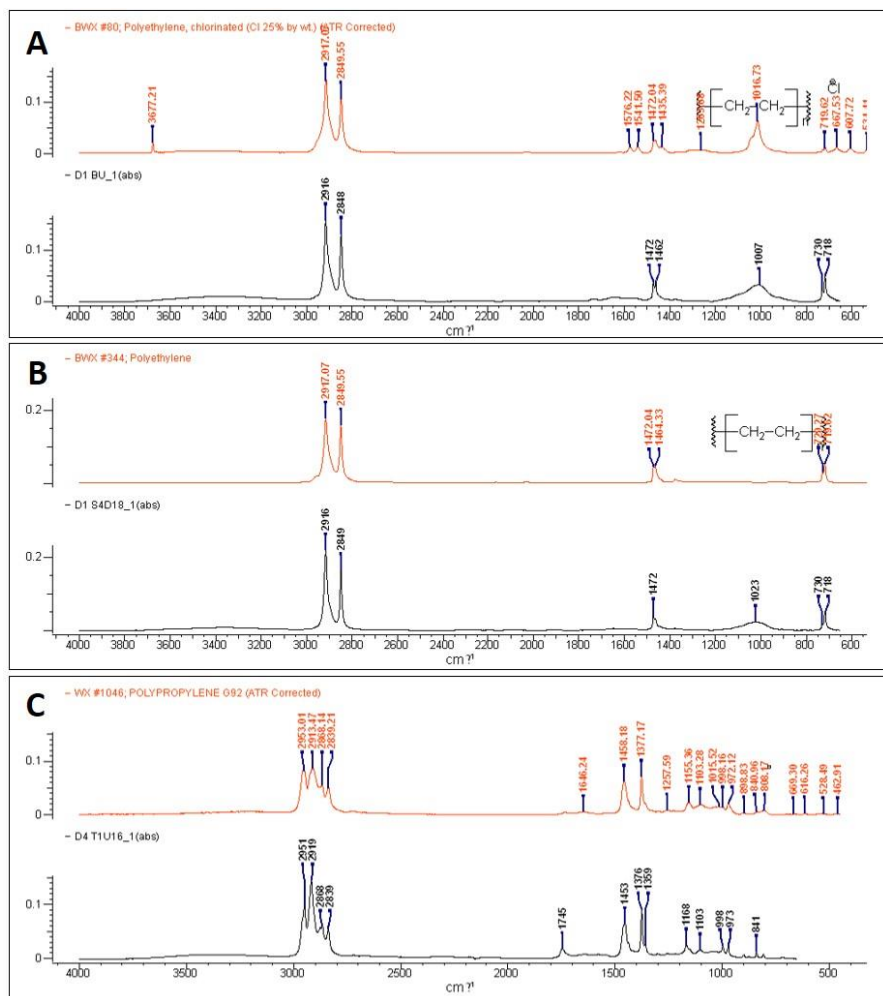


Fig. 3: FTIR spectra of selected microplastics from the surface water of Laguna de Bay. A. Chlorinated Polyethylene; B. Polyethylene; C. Polypropylene.

Attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy of selected microplastic fragments (Fig. 3) revealed the following plastic materials: polyethylene; polyethylene foam; medium-density polyethylene; high-density polyethylene; polyethylene ionomer; chlorinated polyethylene; polypropylene; maleic anhydride grafted polypropylene; styrene-butadiene isoprene rubber; styrene copolymer phosphonic diacid monomer; modified polyvinyl chloride with nitrile rubber; acrylonitrile butadiene copolymer. The ATR-FTIR configuration was set to 16 scans and 4 cm^{-1} resolution.

These polymer materials can be found in plastic products such as grocery bags, rubber tires, appliances, and plumbing products. Since Laguna de Bay is surrounded by various residential, commercial, and industrial establishments, it is difficult to avoid microplastic pollution, and it is polluted by microplastics as provided with the data of this study.

CONCLUSION

The study reports the microplastics' occurrence and profile isolated in Laguna de Bay. Fewer microplastics were isolated in Brgy. Napindan is compared to another sampling since it is located at the source of the Pasig River. The sites in Brgy. Sampiruhan and Brgy. San Isidro had higher microplastic occurrences, which could be attributed to the surrounding urbanized communities. The isolated microplastics had lengths ranging from 500 to 2,000 μm and appeared round, ranging from 0.2 to 0.8, and varied between sampling sites. The color coordinates of the microplastics suggest yellowing, an apparent sign of photodegradation of the plastic polymer and its additives. This study was also carried out to investigate the state of microplastics in Laguna de Bay, as there has been less focus on this area despite the obvious importance of the lake in agriculture.

RECOMMENDATIONS

Effective management programs for plastic pollution are recommended in the study sites and adjacent areas. In addition, it is suggested that pollutant accumulation in freshwater ecosystems in other provinces nearby should be further investigated to ensure environmental and human health safety conditions. It is also highly recommended that the agencies related to Laguna de Bay enforce policies that would reduce microplastic pollution and improve their management of the issue. Agencies such as Laguna Lake Development Authority (LLDA), Department of Energy and Natural Resources (DENR), Department of Trade and Industry (DTI), and National Water Resources Board may refer to this study on how to mitigate the growing problem of microplastics in Laguna de Bay.

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SUPPLEMENTARY DATA

The data gathered and used in the study was uploaded in Mendeley Data (URL: https://data.mendeley.com/datasets/bxd3k2yntp/1?fbclid=IwAR3Z3IrSFjf_vIB_U_DAh9MInzwYDD9UOkJszndgKgCaKi1D0GvhMykr6N8). The data in Mendeley is available to the public for academic purposes.

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