

Preliminary determination of microplastics in bivalves collected from Phu Yen, Central Viet Nam

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Abstract. In this study, four common bivalves including *Meretrix lusoria* (clam), *Anadara subcrenata* (ark clam), *Crassostrea gigas* (oyster), and *Perna viridi* (green mussels) which are popularly consumed were collected from a seafood market in Phu Yen, Central Viet Nam to investigate the occurrence of microplastics (MPs) in their tissues. Samples were treated with a 10 % (w/v) KOH solution for 48 hours and incubated at 40 °C. The base-sample ratio was 10:1 v/w (i.e 10 mL of 10 % KOH solution per 1.0 gram of a tissue sample). Sodium iodide was used to extract MPs from tissue samples. The number of MP items determined in clam, ark clam, oyster, and green mussels registered by weight and individual were 0.2 - 0.5, 0.1 - 0.8, 0.1 - 0.5, and 0.1 - 1.0 (item/g-ww), and 1 - 3, 1 - 3, 1 - 9, and 1 - 5 (items/individual), respectively. Fiber has the common shape (69 - 92 % of total items), followed by fragment. Furthermore, polyethylene terephthalate was the most common fiber, which was often confirmed when microfiber was analyzed by the FTIR technique. As the first investigation on MPs in bivalve tissue from Phu Yen, Central Viet Nam, this study indicated that relatively high levels of MPs were found in commercial bivalves collected from a seafood market in Central Viet Nam, warning of a potentially high risk to humans when consuming bivalves as daily food.

Keywords: microplastics, bivalves, Phu Yen, central Viet Nam.

Classification numbers: 3.2.1, 3.6.1.

1. INTRODUCTION

It is estimated that more than 5 trillion pieces of plastic (about 250,000 tons) have been floating in the world's oceans [1]. According to the 2017 - 2018 annual review report of Plastic

Europe, 8.4 million tons of plastic waste were additionally deposited into the marine environment due to the increasing production and consumption of plastic worldwide [2]. A large amount of plastic debris of continental origin enters the marine environment primarily through rivers [3], industrial and urban wastewater, and runoff from beach sediments and adjacent fields [4]. Other sources of plastic debris in the marine environment are direct sources, including offshore industrial activities (such as oil and gas exploration), aquaculture activities, losing fishnets, and littering at sea including tourism activities [4].

Microplastics (MPs) are small particles (less than 5.0 mm), containing non-biodegradable, and persistent polymers, which are ubiquitous in the environment and raise many questions to the public worldwide because of their great impacts on health, biodiversity, and ecosystem functioning [5 - 11]. Moreover, some studies indicated that MPs in the environment can act as media for adsorbing heavy metals or micro-pollutants, which are more toxic [12, 13].

MPs were found in mussels in five European countries (France, Italy, Denmark, Spain, and the Netherlands) [11]. Additionally, 3 - 5 items/10 g of samples were found in commercial mussels collected from Belgium [14]. Other studies on MPs in commercial bivalves in China also reported that the average number of MPs (size range of 5 - 5000 μm) was 2 - 11 items per sample or 4 - 57 items per individual [15]. In Viet Nam, a high rate of economic growth coupled with improper solid waste management leads to an extremely high potential for MPs pollution. However, limited information regarding the occurrences of MPs in environmental matrices has been reported in Viet Nam. Therefore, studying on occurrences of MPs in environmental matrices and sea creatures is necessary, which will provide useful information for enhancing public awareness of the pollution of MPs and acting to reduce MP discharges into the environment.

A previous study suggested that Viet Nam has the world's fourth largest amount of plastic waste that originates from the land and is deposited into the ocean with 1.83 million metric tons per year [16]. High levels of plastics and MPs were found in the Saigon River, which flows through Ho Chi Minh City - one of the most developed cities in Viet Nam [16]. The results of daily analysis of solid waste collected from the Saigon River indicated that the amount of plastic debris accounts for 11 - 43 % of total solid waste floating on the river. Furthermore, it was estimated that each person daily discharges from 0.96 to 19.91 g of plastic debris into the river, which means that every person yearly releases from 350 to 7270 g of plastic into the river.

Bivalves are popular seafood that widely lives in coastal waters of Viet Nam. In addition, humans have the habit of eating all of the soft parts of bivalves when consuming them as seafood. Therefore, a high risk to human health might be posed when people eat bivalves containing MPs with or without adsorbed contaminants on the particles [18, 19]. Surprisingly, aquaculture products can also be contaminated by MPs due to the consumption of food containing MPs [20]. Therefore, through the food chain, MPs along with toxins adsorbed on MPs can enter and accumulate in higher-level animals and eventually in humans [18, 19, 21 - 23].

In this study, four popularly consumed bivalves species were collected from a seafood market in Phu Yen, Central Viet Nam. The abundance and types of MPs were measured in each sample. This study aims to determine if the commercial bivalves in Central Viet Nam have been polluted by MPs and to distinguish the differences in characteristics of MP pollution among the different species.

2. MATERIALS AND METHODS

2.1. Chemicals and other materials

The materials used in this study included potassium hydroxide (KOH) and sodium iodide (NaI), which were purchased from Sigma-Aldrich. Solutions of KOH (10 % w/v) and NaI were prepared by dissolving powder/pellet in ultrapure distilled water. Filter papers were supplied by Whatman Inc. (No.1821-047).

2.2. Sample collection

A total of sixty bivalves samples including four species of marine bivalves (Table 1) were randomly bought from the biggest seafood market in Phu Yen, Central Viet Nam in January 2021. All samples were kept in cool containers with ice during transportation to the laboratory and then lyophilized after the removal of the shell. These species were the most commercially popular bivalves including *Meretrix lusoria* (clam), *Anadara subcrenata* (ark clam), *Crassostrea gigas* (oyster), and *Perna viridi* (green mussels) corresponding to 4 genera *Veneridae*, *Arcidae*, *Ostreidae* and *Mytilidae*, respectively. The samples collected were derived from fishery farms or wild environments along the coastal waters of Central Viet Nam.

Table 1. Length and weight of bivalves from a local market in Phu Yen, Central Viet Nam.

Genus	Species	Number	Shell length (cm)	Shell width (cm)	Shell weight (g/individual)	Soft tissue weight (g/individual)
<i>Veneridae</i>	<i>Meretrix lusoria</i> (clam)	12	1.6 ± 0.2	1.3 ± 0.2	15.0 ± 2.2	7.2 ± 2.3
<i>Arcidae</i>	<i>Anadara subcrenata</i> (ark clam)	12	5.4 ± 0.2	3.7 ± 0.1	33.0 ± 10.0	8.3 ± 3.4
<i>Ostreidae</i>	<i>Crassostrea gigas</i> (oyster)	12	9.2 ± 1.2	5.9 ± 0.7	74.7 ± 6.6	11.2 ± 3.6
<i>Mytilidae</i>	<i>Perna viridi</i> (green mussel)	12	8.2 ± 1.1	3.6 ± 0.4	23.8 ± 6.6	6.4 ± 1.9

2.3. Samples treatment

Firstly, the size and weight of bivalve shells were recorded. The shells were then opened, and the tissue content inside was moved into a 125 mL glass flask with a stopper capacity. Individually, the tissue of *Crassostrea gigas* was separated into two fractions due to its greatest weight, then the MPs found in two fractions were combined into one result.

Based on a previous study [24] and our investigation [25], a solution of 10 % KOH was added to each flask to digest the organic matter depending on the weight of the soft tissue in each flask (10 mL of 10 % KOH solution per 1.0 gram of a tissue sample). The flasks were covered and placed in an oven at 50 °C for 48 hours and then at ambient temperature for 24 - 48 hours depending on the digestion effect of the soft tissue.

2.4. Flootation and filtration with sodium iodide

The digested sample was mixed with NaI solution of appropriate concentration (2 M) to separate other high-density materials from the digestate of the whole tissue before microscopical inspection of the filter membrane. The supernatant was separated and passed through the filter funnel, the remaining pellets were continued to dissolve with NaI, then to separate the supernatant through the filter membrane, repeating this process until all the polymers were removed from the undissolved portion [23]. Each flask was added with 30 mL of filtered NaI solution, ultrasonically shaken for 60 minutes, and then allowed to settle overnight. The supernatant was directly filtered through a Whatman GF/B glass microfiber filter (1.0 μm in pore size, 47 mm in diameter).

2.5. Quality control of analysis

Blank samples were processed at the same time as field samples to correct the possible contamination during pretreatment and analysis. Chemical solutions (2 M NaI and 10 % KOH) and DI water were filtered with 4.7 mm filter paper ($d = 1.0 \mu\text{m}$) before the contamination was eliminated. All instruments were kept clean throughout the process by washing with filtered DI water and covering until used. During the processing of the samples, the contamination with airborne microplastics was prevented and the procedural blanks only contained 0.2 ± 0.4 items/filter.

2.6. Observation of microplastic and chemical composition analysis

The size/type/color of MPs on filters was observed under an Olympus BX51 microscope attached with camera Infinity 1. A visual assessment was applied to identify the types of microplastics according to the physical characteristics of the particles. Microplastic components and structures were identified by an iS50 FTIR system with a built-in Attenuated Total Reflection (ATR), diamond crystal. In this study, only large-size fibers ($\geq 50 \mu\text{m}$) could be taken out from the filter and used for individual analysis by ATR-FTIR. The chemical type of the found polymers could be identified based on comparing the obtained characterized absorption bands of FTIR spectra to those of the standard polymers.

3. RESULTS AND DISCUSSION

3.1. Abundance of microplastics in bivalves

The average number of MPs found in tissues varied from 0.2 to 0.5 items/g (wet weight) and from 1.4 to 3.0 items/individual of bivalves (Figure 1). *Perna viridi* (green mussel) contained an average of 0.5 ± 0.3 item/g-ww and showed the highest levels of MPs contamination by weight (Figure 1A). One-way ANOVA revealed a statistically significant difference in MPs concentration in *Meretrix lusoria* (clam), *Anadara subcrenata* (ark clam), *Crassostrea gigas* (oyster), and *Perna viridi* (green mussels) registered by weight, demonstrating that they can be considered as independent groups ($p < 0.05$). Pairwise comparison of means with equal variances indicated the significance of statistical independence in MPs between the green mussel and ark clam ($p < 0.05$), green mussel and clam ($p < 0.05$). The results suggest that different living regions of four species strongly influence the accumulating level of MPs in bivalves.

On the other hand, oyster showed the highest microplastics as 3.3 ± 2.4 items per individual (Figure 1B) because the size of this specie is the largest. Similarly, when comparing MPs levels in these four species by individual, pairwise multiple comparison indicates that MPs in oyster are significantly higher than that of clam and ark clam, respectively ($p < 0.05$).

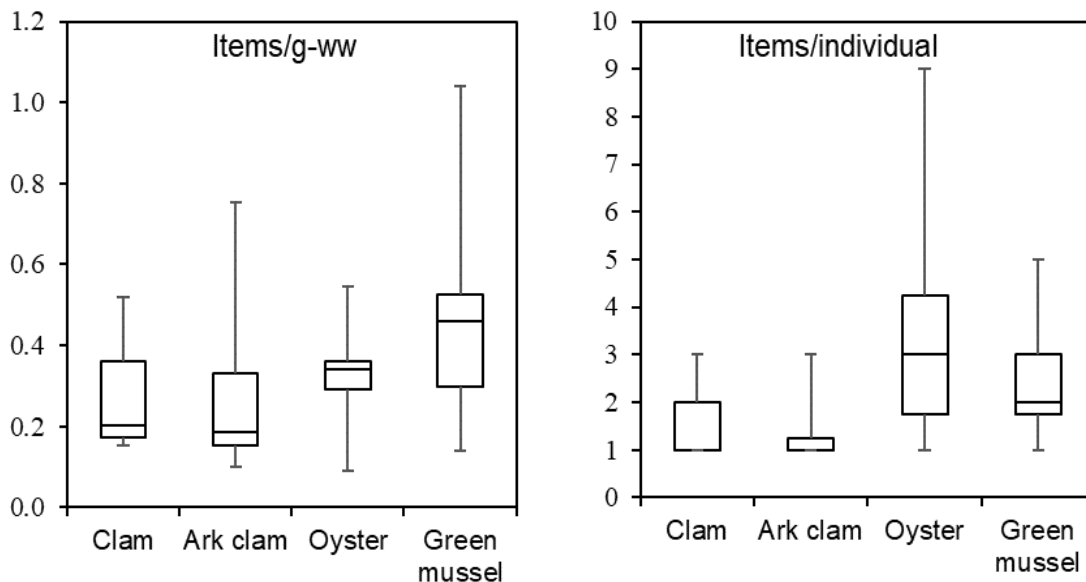


Figure 1. Abundance of microplastics in bivalves from a seafood market in Central Viet Nam (12 replicates were set for each specie).

Table 2. Comparison of microplastic pollution in bivalves in the present study with those in previous studies.

Samples (nation)	Digestion method	Levels of MPs (Items/individual)	size (μm)	Structure analysis method	Reference
Blue mussels (France)	KOH 10 %; m/v	2.8 ± 1.3	30 - 200	FT-IR	[26]
Green mussels (Vietnam)	KOH 10 %; m/v	2.9		$\mu\text{FT-IR}$	[27]
Mussels (Europe)	20 mL HNO_3 69 % (overnight) 7 5°C	0.2 - 1.2	-	Raman	[10]
Commercial mussels (China)	200 mL H_2O_2 30 %, at 60°C	4.3 - 57.2	< 250	$\mu\text{FT-IR}$	[15]
Bivalves (Germany)	20 - 25 mL HNO_3 69 %	0.36 - 0.46	< 50	Raman	[11]

Table 2 compares the results obtained in this study with those reported in previous studies, which indicates that the levels of MPs found in this study are relatively higher than those reported for MPs in mussels collected in five European countries with the highest number of pieces reaching 1.2 items per mussel [10]. The levels found in this study are similar to that observed by De Witte *et al.* [14] for commercial mussels from Belgium with 0.3 - 0.5 items/g of samples. Phuong *et al.* [26] also reported the MPs levels in blue mussels in France which were

similar to our research results. Another study on MPs in commercial bivalves in China also reported that the average number of MPs was 2 - 11 items/g of samples or 4 - 57 particles/individual [15], which was approximately one order of magnitude higher than that in our study. Interestingly, the MPs found in green mussels in our study were a little bit higher than those reported by Phuong *et al.* [27] for green mussels collected from Thanh Hoa Province, while the sample sizes in the two studies were similar.

3.2. Shape of microplastics in bivalves

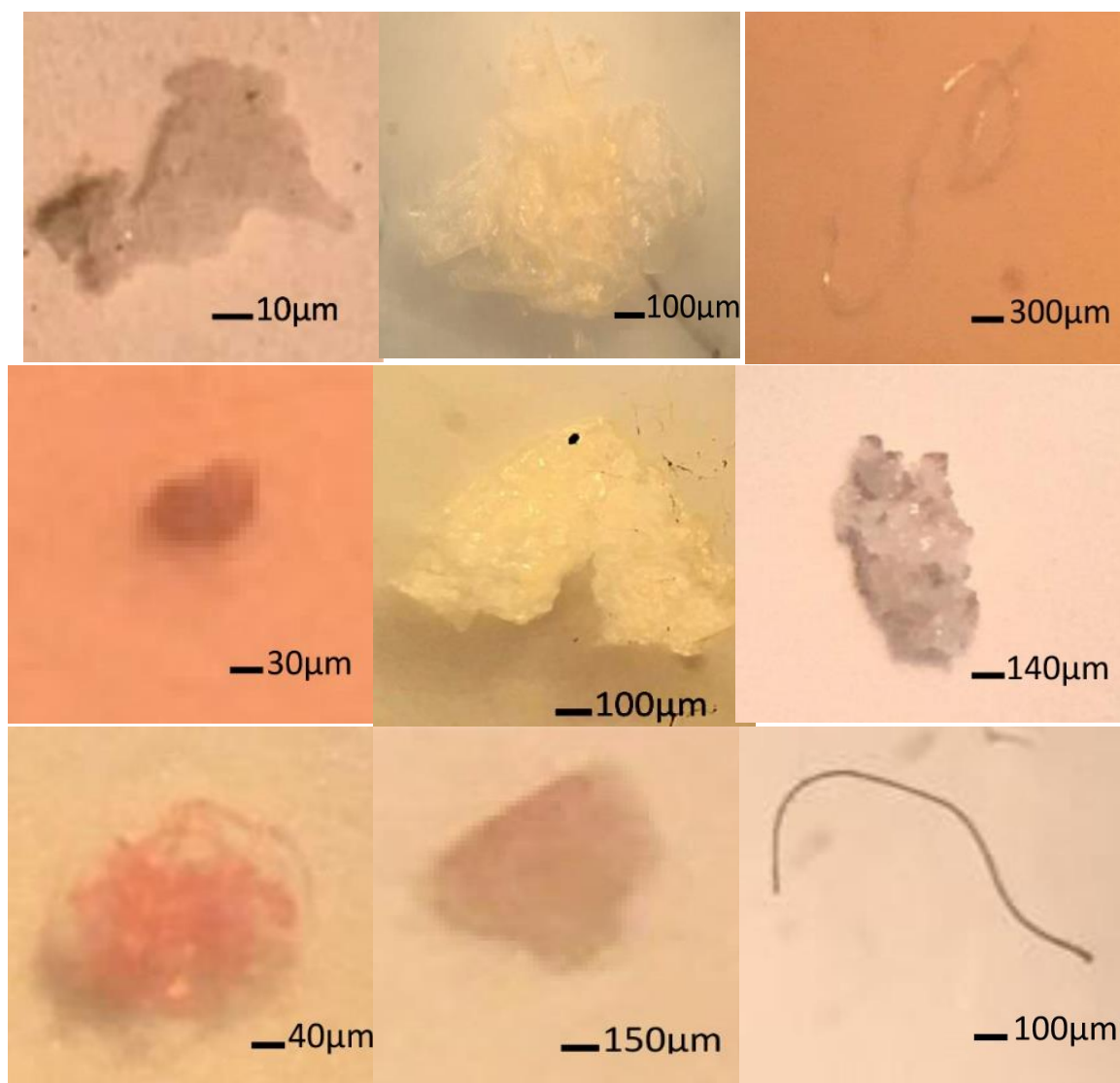


Figure 2. Different shapes of microplastics in bivalves collected from a seafood market in Central Viet Nam. The photographs were taken directly on the filter paper.

Figure 2 presents different shapes of MP, including fibers and fragments, found in the tissue of bivalves. The most diverse shape was observed in fibers (69 % - 92 %), followed by

fragments (8 % - 31 %), while pellet was not observed in all samples. As shown in Figure 2, popular colors of fiber and fragment were black, white, red, blue, and transparent.

The shape distributions of MPs in four species of bivalves (Figure 3) indicate that fiber is the predominant shape in all species, accounting for from 69 % to 92 % of total MPs. Similar results were observed by De Witte *et al.* [14], Li *et al.* [15] and Mathalon and Hill [28]. Fibers were not reported in Van Cauwenberghe *et al.* [10] and Van Cauwenberghe and Janssen [11] due to the damaging effect of concentrated HNO₃ on fibers during the digestion process of microplastics. The fiber MPs might originate from the plastic rope used in fishing nets or plastic lines where farmed bivalves are grown.

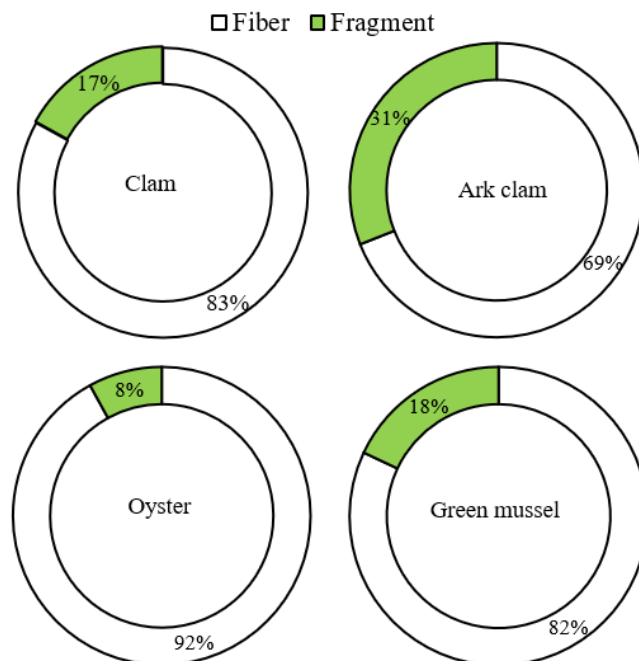


Figure 3. Composition of the different shapes of microplastics in bivalves from a seafood market in Central Viet Nam. Twelve replicates were set for each species.

3.3. Identification of microplastics in bivalves with ATR-FTIR

In this study, the FTIR spectrum of microfibers (in the red and blue lines) is frequently matched with the FTIR spectrum of PET standard. The characteristic bands of PET were 1713 cm⁻¹ corresponding to C=O stretching vibration in ester and the large bands at 1243 cm⁻¹ and 1095 cm⁻¹ could be assigned to C–O–C stretching one in ester. Besides, characteristic band of 1016 cm⁻¹ could be identified as in-plane vibration of the aromatic ring, and the narrow band at 725 cm⁻¹ could also be assigned to CH₂ bending-out of plane vibration of aromatic ring [29, 30]. A fairly good comparison between spectrum of the found microfiber and PET standard can be observed in Figure 4. This result suggested that PET might be the dominating microplastic one accumulated in four common bivalves investigated in this study. However, further studies should be conducted for more accurate identification of which polymer could be considered dominant in bivalves caught in our marine investigated area, when all microplastic particles will be introduced to better technique (micro-FTIR, micro-Raman) for measurements towards the aimed chemical composition determination.

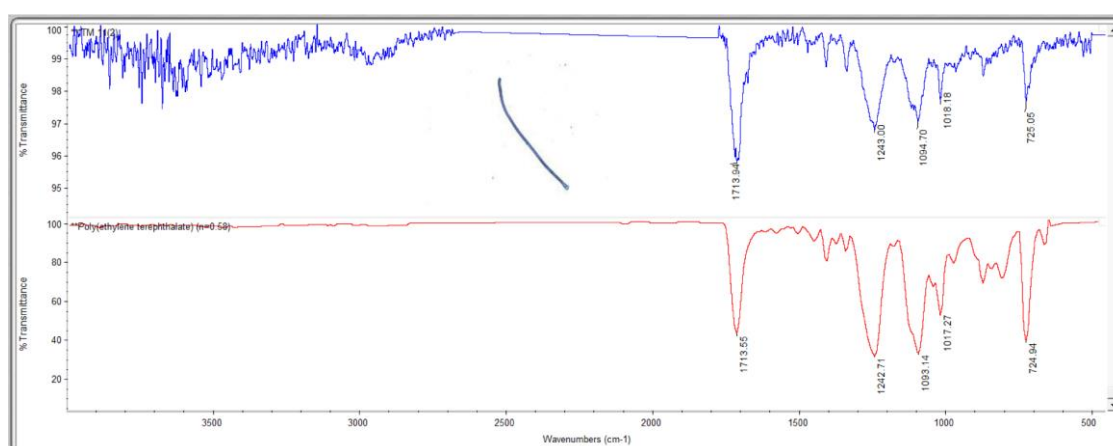


Figure 4. The FTIR spectra of a common MP fiber found in samples with high similarity with polyethylene terephthalate (the red and blue lines are the FTIR spectra of standard and sample, respectively).

4. CONCLUSION

As one of the first investigations on MPs in bivalves in Central Viet Nam, this study successfully separated and evaluated the occurrences of MPs in bivalve tissues collected. The results indicated that a relatively high level of MPs was found in the commercial bivalves collected from a seafood market in Central Viet Nam, suggesting that the occurrence of MPs in seafood is unneglectable and a potentially high risk to humans consuming bivalves as daily food is posed. Fibers were the most popular shape of MPs, indicating some possible sources of MPs in bivalve tissues. In addition, the type of polymer found in samples was also preliminary identified via FTIR spectrum, which reveals that polyethylene terephthalate is a common polymer constituting micro-fibers in MPs found in the collected samples. Further studies should be conducted to determine if features of species or the pollution levels of the surrounding environment cause such specificity in bivalves in particular and in seafood in general.

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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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