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Fate and transport of microplastics from water sources

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Microplastics as environmental pollutants affect surface water and groundwater. Surface water, groundwater and branded drinking water bottles were analysed in and around Chennai, Tamil Nadu, India. The total count of microplastics was found to be 66 particles with fibrous and fragmented shape, colours such as white, blue, green, yellow, pink and black under optical microscope. SEM-EDX-used to study morphology and elemental analysis of microplastics confirmed the presence of heavy metals such as Cr, Ti, Mo, Ba and Ru adhered to their surface. Polyethylene terephthalate and polyamide were confirmed by the presence of functional groups of the polymers by FTIR equipped with attenuated total reflectance.

Keywords: Microplastics, heavy metals, pollution, polyamide, water sources.

PLASTICS are synthetic organic polymers formed by the process of polymerization[1]. The use of plastics has increased worldwide and the annual production is around 322 million metric tonnes[2]. In India, approximately 5.6 million tonnes (mt) of plastic waste is generated annually[3]. Plastic debris which is less than 5 mm is referred to as microplastic, and categorized as primary and secondary. The main sources of microplastics in the marine environment are land and sea-based litter[4]. Microplastics are a big threat to marine organisms as they are ingested by them. A study was done on the distribution, weathering and chemical characteristics of microplastics on the beaches of Goa, India during the southwest and northeast monsoon seasons[4]. The distribution and characteristics of microplastic pollution along the coast of Chennai, Tamil Nadu (TN), India during pre- and post-flood were also studied[5]. Presence of microplastics and their distribution and characteristics were reported from Rameswaram Coral Island, TN, India[6], marine water from Kuala Nerus and Kuantan Port in Malaysia[7], drinking water treatment plants in Germany[8], freshwater resources like Vembanad lake in Kerala[3], Huron Lake in Canada[9], and three urban estuaries in China[10]. Microplastics were also identified in bottled drinking water, because of the packaging materials (polyethylene terephthalate), which are consumed by humans[11]. In India, only a few studies are available on microplastics contamination in sediment samples of

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Location	Sample type	Microplastics	Reference
Chennai coast	Sediment	Polyethylene and polypropylene	5
Goa coast	Sediment	Polyethylene and polypropylene	4
Coast of Chennai and Tinnakkara Island	Sediment	Not identified	16
Vembanad lake	Sediment	High-density polyethylene, low-density polyethylene, polypropylene and polystyrene	3
Rameswaram Coral Island	Sediment	Polypropylene, polyethylene, polystyrene, polyvinylchloride and nylon	6
Five coastal areas of Tamil Nadu	Sediment	Polyethylene, polypropylene, polyamider, polyester and polystyrene	17

Figure 1. Microplastic contamination reported in India.

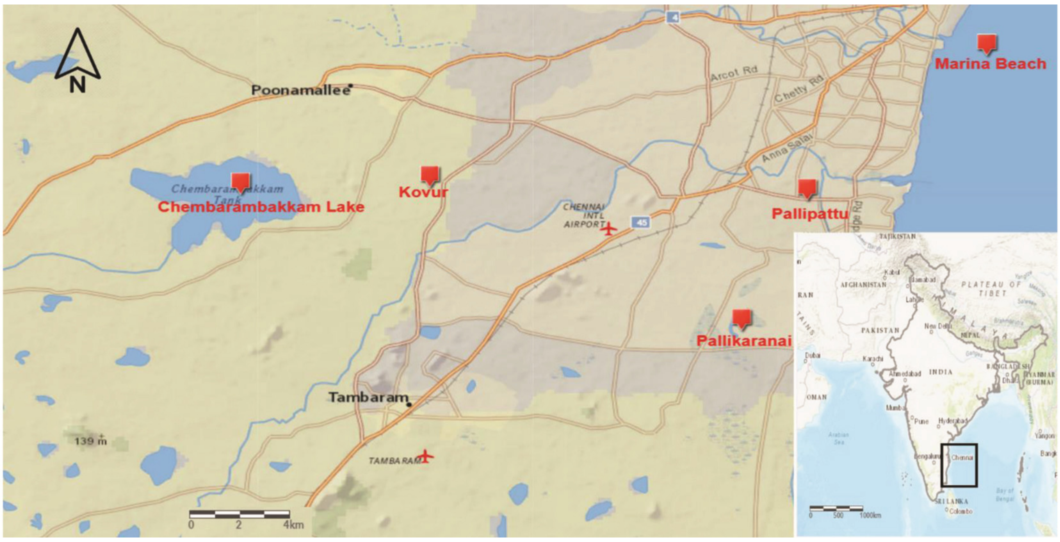


Figure 2. Sampling location in Chennai, Tamil Nadu, India[18].

marine and freshwater/estuaries, and no study has addressed the presence of microplastics in groundwater and packaged drinking water. Hence, the present study was conducted in and around Chennai, to identify microplastics from various sources of water. Figure 1 provides a summary of microplastic contamination in India.

Chennai is situated along the Bay of Bengal coast and its total population is around 10 million. Water samples were collected during March 2019 from different places like Marina seawater (M), Pallikaranai (P) and Kovur (K) groundwater, Chembarambakkam Lake (CL) surface water which is the drinking water source for Chennai city, Pallipattu from where the water (groundwater) is supplied for domestic purposes (PP), can water from domestic suppliers (C; 25 l) and branded drinking water bottles (B1, B2, B3, B4 and B5). Surface and groundwater samples were collected in glass bottles and taken directly to the laboratory for further examination. Figure 2 shows the sampling location.

Equal volume (1 litre) of water from each sample was filtered using vacuum filtration set-up with 0.45 µm Whatman cellulose nitrate filter paper and dried at room temperature for further analysis. The dried filter paper containing the microplastics was placed under an optical microscope at 40× magnification (Labomed LX-300, USA), equipped with a digital camera. Particles identified as microplastics were analysed for physical characteristics such as shape, colour and count. Morphological and elemental analyses of the particles were performed using a scanning electron microscope (SEM) equipped with an energy dispersive X-ray (EDX) analyser (Hitachi S-3400, USA). EDX analysis was performed at low vacuum with an operating voltage of 15 kV. Microplastics were then examined using Fourier transform infrared spectroscopy (FTIR) equipped with attenuated total reflectance (JASCO 6600typeA, Easton, USA).

The number of microplastics was found to be 66 from eleven water samples. Fibrous and fragment shaped microplastics were found, with fragment shaped ones being dominant. White, green, yellow, brown, blue, pink and black were the colours identified. Figures 3 and 4 show the colour and count of microplastics present in each sample. Figure 5 highlights the factors causing microplastic contamination.

Table 1. Nature of microplastics and their confirmation

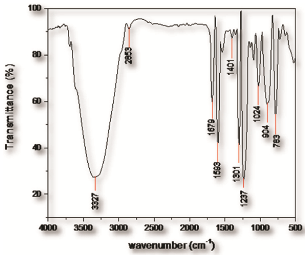
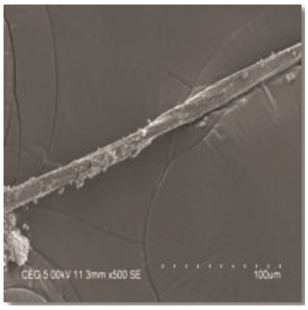
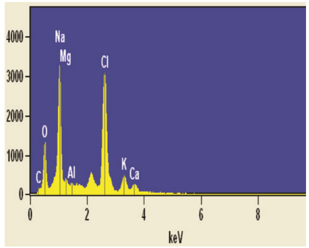
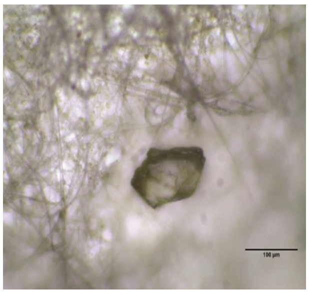
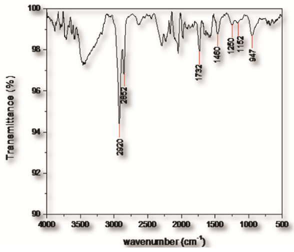
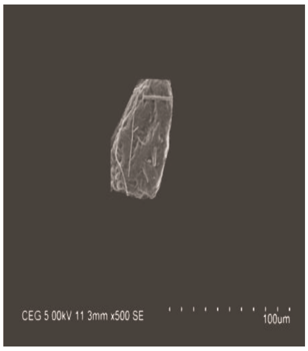
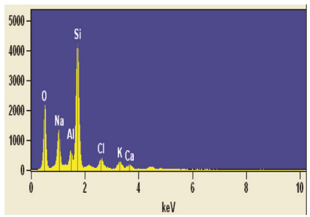
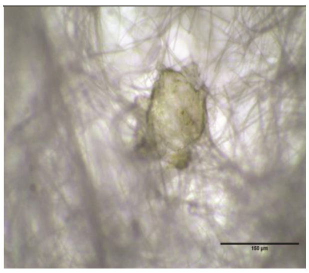
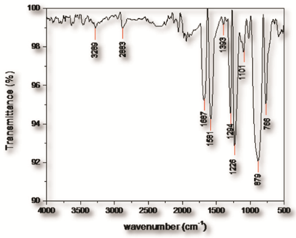
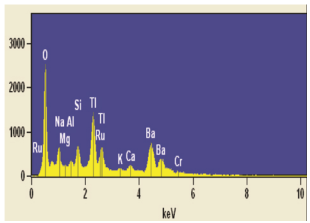
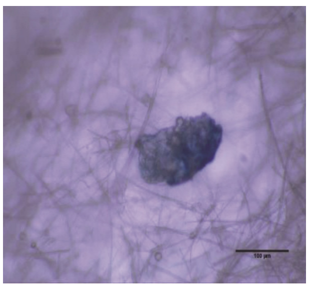
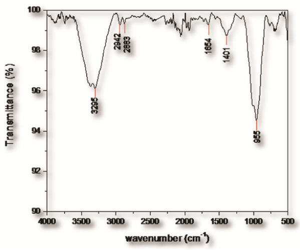
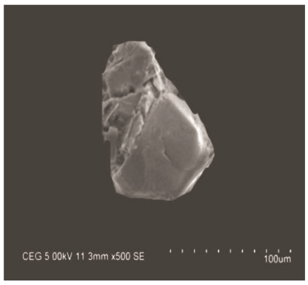
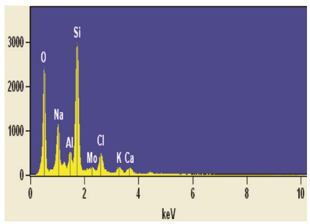
Sample Id	Dominant microplastic image	Fourier transform infrared spectroscopy	Scanning electron microscope	energy dispersive EDX
Surface water				
M				
CL				
Ground water				
PP				
P				

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Table 1. (*Contd*)

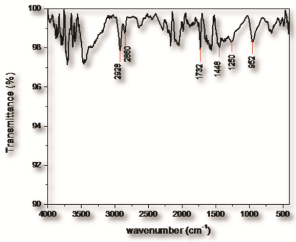
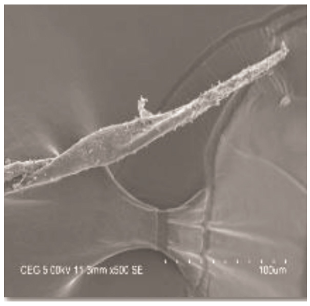
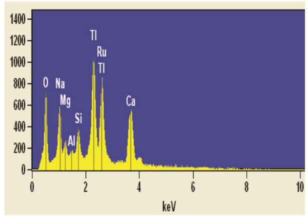
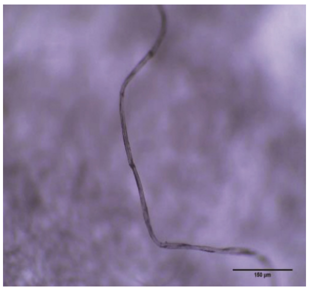
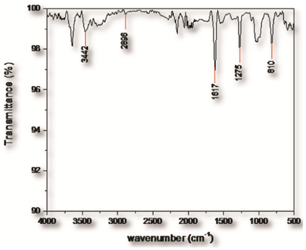
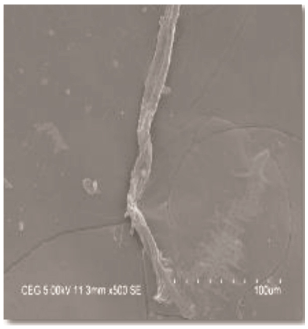
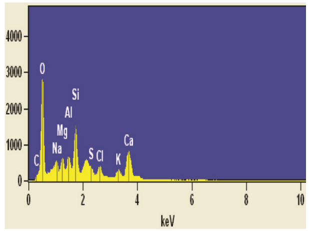
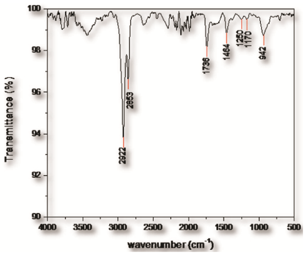
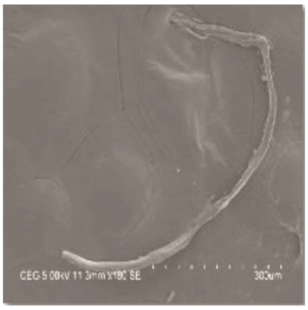
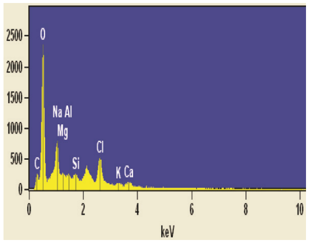
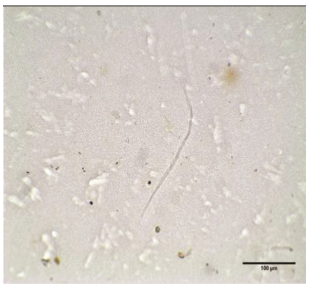
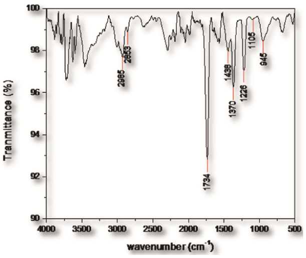
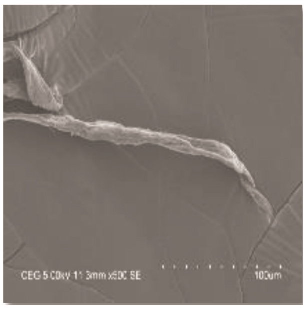
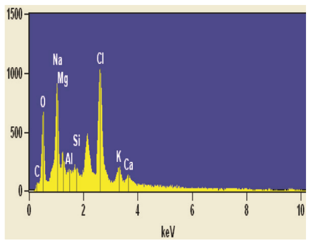
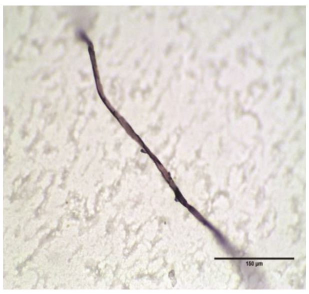
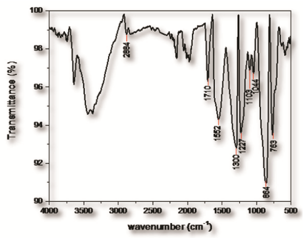
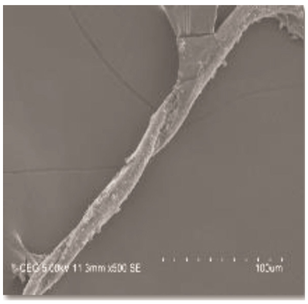
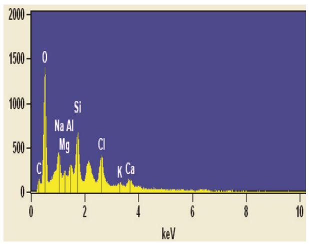
Sample Id	Dominant microplastic image	Fourier transform infrared spectroscopy	Scanning electron microscope	energy dispersive EDX
K				
Commercial drinking water				
C				
B1				
B2				
B3				

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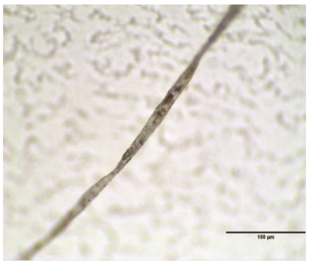
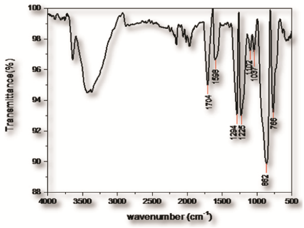
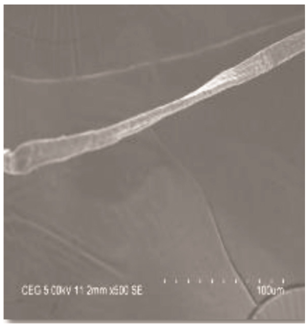
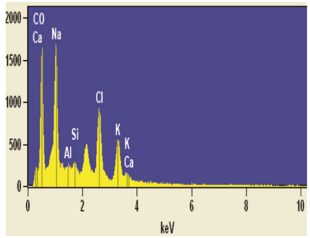
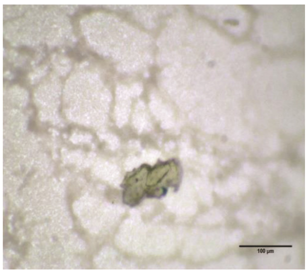
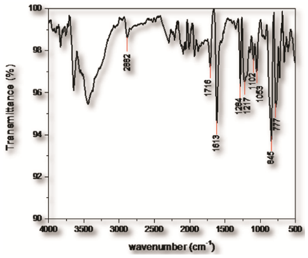
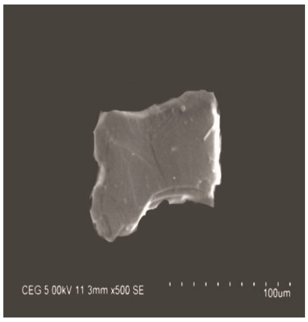
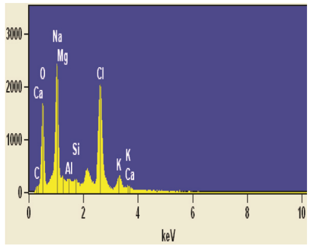
Table 1. (*Contd*)

Sample Id	Dominant microplastic image	Fourier transform infrared spectroscopy	Scanning electron microscope	energy dispersive EDX
B4				
B5				

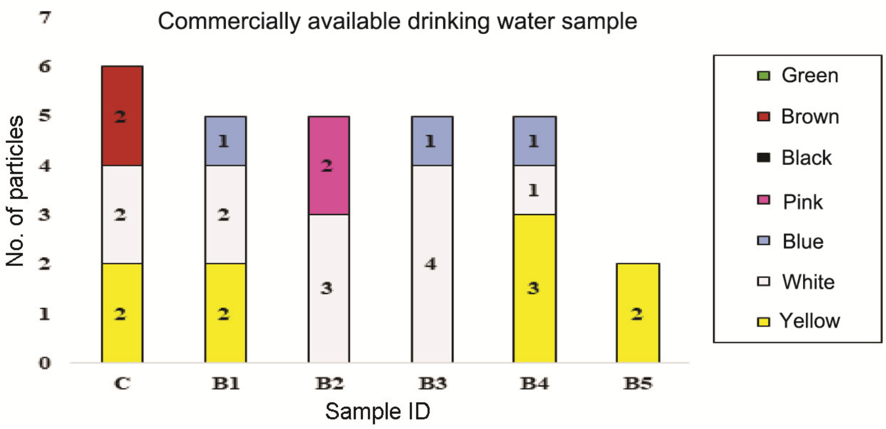


Figure 3. Colour classification of microplastics in commercially available drinking water.

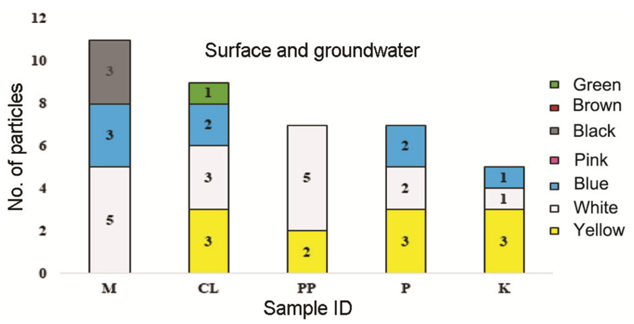


Figure 4. Colour classification of microplastics in groundwater and surface water.

FTIR confirms microplastics by the presence of functional groups of the polymer. For polyethylene terephthalate, the absorption bands were observed at C=O stretch around 1716 cm^{-1}, CH_2 stretching around 2882 cm^{-1}, C=C stretch around 1580 cm^{-1} and additional peaks around 745 and 865 cm^{-1} CH_2 bend and C–H bend[12,13]. Polyethylene terephthalate was found in the samples of branded water bottles, Chembarambakkam and Kovur. The N–H stretching around 3442 cm^{-1}, 2942 CH_2 asymmetric and symmetric stretch and 2883, C=O stretch around 1654 cm^{-1}, NH bend and CH stretch around 1581 cm^{-1} and 1226 cm^{-1} respectively, confirms the

Sample Id	No. of particles/litre	Factors causing contamination
Surface water		
M	11	Fishing, floods, transportation and human activity[17,19].
CL	9	Dumping of household and various industrial plastic waste[17].
Groundwater		
PP	7	Located in the canal bed of River Cooum, Chennai[17].
P	5	Dumping yard for solid waste[15].
K	4	Urbanization[10].
Commercial drinking water		
C	6	Improper purification system and degradation of plastic parts used in the system[11].
B1	5	
B2	5	
B3	5	
B4	5	
B5	2	

Figure 5. Factors causing microplastic contamination.

Table 2. Presence of heavy metals adsorbed on the surface of microplastics

Sample Id	C	O	Na	Mg	Al	Si	Cl	K	Ca	S	Rn	Cr	Tl	Ba	Mo
Surface water															
M	✓	✓	✓	✓	✓	✓	✓	✓	✓						
CL		✓	✓		✓	✓	✓	✓	✓						
Groundwater															
PP		✓	✓	✓	✓	✓		✓	✓		✓	✓	✓	✓	
P		✓	✓		✓	✓	✓	✓	✓						✓
K		✓	✓	✓	✓	✓			✓		✓				
Commercial drinking water															
C	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓					
B1	✓	✓	✓	✓	✓	✓	✓	✓	✓						
B2	✓	✓	✓	✓	✓	✓	✓	✓	✓						
B3	✓	✓	✓	✓	✓	✓	✓	✓	✓						
B4	✓	✓	✓		✓	✓	✓	✓	✓						
B5	✓	✓	✓	✓	✓	✓	✓	✓	✓						

presence of polyamide[12,13]. Polyamide was present in seawater, Pallikaranai, Pallipattu and can water. Table 1 depicts the nature of microplastics and their confirmation.

SEM-EDX shows the morphology of microplastics and the presence of chemical elements. Elements such as sodium, magnesium, potassium, aluminium, silica, calcium, chlorine and oxygen adhere to the surface of microplastics. Carbon indicates the presence of polymer materials[14]. Heavy metals such as ruthenium, thallium, barium and chromium present in the Pallipattu sample have been contributed by River Cooum. Molybdenum was found in Pallikaranai sample due to the large sewage treatment plant located around the region[15]. Ruthenium and thallium present in Kovur groundwater sample were due to urbanization[10]. Table 2 lists the heavy metals adsorbed on the surface of microplastics.

In India, this is the first report to study the occurrence of microplastics in drinking water sources. The reason for the occurrence of microplastics in water is due to the immense usage of plastics, and there is no proper waste management system. As microplastics are consumed by humans from drinking water, further studies are mandatory to understand the impact of microplastics on humans. For controlling microplastic pollution, the use of plastics must be reduced, or they must be recycled and reused. Proper waste management system and drinking water purification process have to be implemented for the removal of microplastics.

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Transcriptomic analysis of chilling-treated tobacco (*Nicotiana tabacum*) leaves reveals chilling-induced lignin biosynthetic pathways

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Chilling stress is one of the most important environmental stresses for chilling-sensitive species. The present study conducted RNA-Seq and WGCNA analysis to clarify the correlation patterns among genes of different treatments in tobacco (*Nicotiana tabacum*). A total of 10,355 DEGs were found in chilling treatment relative to control treatment. Additionally, functional annotations revealed that 48 genes were found to be specifically expressed in lignin biosynthesis pathway in tobacco seedlings under chilling stress. Our results revealed that the biosynthesis of caffeoyl-CoA was regulated by *HCT* and *C3H*. Furthermore, the G-type lignin biosynthesis branch was enhanced under low temperature, which contributed to an increase in lignin content and changes in lignin composition, indicating that G-type lignin may play an important role in tobacco's resistance to chilling stress.

Keywords: Chilling stress, lignin biosynthesis, *Nicotiana tabacum*, transcriptomic, WGCNA.

CHILLING stress is one of the environmental factors that restrict plant growth and geographical distribution. Plants have evolved a number of sophisticated mechanisms to rapidly respond to changes in the environment and different types of defense mechanisms that protect plants from chilling stress. The synthesis of compounds in the phenylpropanoid pathway (Supplementary Figure 1) fulfills a wide range of such functions, including plant development and interactions with the environment[1].

The phenylpropanoid metabolic pathway mainly starts with phenylalanine (Supplementary Figure 1) and provides the precursors of lignin, which is quantitatively the second most common biopolymer on Earth, following cellulose. The intermediates and products of the phenylpropanoid pathway regulate tobacco growth and

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