Abundance of Biological Ice Nucleating Particles in the Mississippi and Its Major Tributaries

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Abstract: Ice nucleating particles (INPs) are rare among atmospheric aerosols. However, through their ability to induce freezing of cloud droplets in cold clouds, they affect cloud lifetime, cloud albedo, and the efficiency and distribution of precipitation. While terrestrial sources of INPs are the focus of much research, the potential of rivers and lakes to be significant INP reservoirs has been neglected. In the first survey of a major river system, surface waters from the Mississippi, Missouri, Platte, and Sweetwater Rivers, all draining east and south from the Great Divide in the United States of America (USA), were tested for their INP concentrations. The survey comprised 49 samples, taken approximately every 150–250 km along 90% of the Mississippi (from Natchez, MS to the source at Bemidji, MN), the full length of the Missouri, 90% of the North Platte, and all of the Sweetwater. Samples were analysed using the immersion freezing method. The highest freezing temperature varied between -4 and $-6\,^{\circ}\text{C}$, and the concentration of INPs active at $-10\,^{\circ}\text{C}$ or warmer ranged from 87 to 47,000 mL^{-1}. The average INP concentration at $-10\,^{\circ}\text{C}$ was 4950 mL^{-1}, almost four orders of magnitude greater than the numbers of INPs typically found active at this temperature in seawater. The majority of INPs (69 to >99%) were heat labile (deactivated by heating to $95\,^{\circ}\text{C}$) and therefore likely to be biological. Although the surface area of rivers is limited, their significant concentrations of INPs suggest that freshwater emissions should be investigated for their potential impact on regional cloud processes.

Keywords: ice nucleating particles; Mississippi; Missouri; Platte; rivers; freshwater; regional atmospheric dynamics

1. Introduction

Ice nucleating particles (INPs) form only a very small subset of all aerosols in the atmosphere [1]. Even so, warmer than about $-38\,^{\circ}\text{C}$, INPs are required to catalyse the formation of ice crystals in clouds in a process termed heterogeneous nucleation. Colder than $-38\,^{\circ}\text{C}$, INPs are not required, and this is termed homogenous freezing. The action of the INPs greatly influences the distribution of liquid and ice particles within clouds. They thereby exert a potentially strong modulation of Earth's energy budget and the water cycle through their modification of cloud radiative properties, the release of latent heat, and the efficiency and distribution of precipitation [2–5].

Terrestrial sources of INPs have received much attention. In addition to ongoing interest into the action of both mineral dusts and soot [6–8], the role of the soil organic matter, which includes INPs produced by the decomposer community, as a source of atmospheric INPs is now being more closely examined [9–12]; pioneering work identified the litter layer, in particular, as a prodigious source of biological INPs in diverse global ecotypes [13,14]. By contrast, while oceans comprise 71% of the Earth's surface area and emit INPs within sea spray—mainly by bubble bursting—they

are only now being investigated more systematically [15–21]. Until recently, the only systematic study of INPs in the marine boundary layer was that undertaken by Bigg [22]. Low INP levels over oceans and variable production of biological INPs has recently been documented in marine sea spray aerosol [18,23], and higher levels appear to be generated by planktonic blooms or the ensuing microbial succession [16,18,19,24,25]. Their emission from the ocean surface will likely be enhanced by their enrichment in the sea surface microlayer [17,26].

Freshwaters as sources of INPs have been largely overlooked. Morris et al. [27,28] showed cultivable *Pseudomonas syringae* were present in a variety of fresh water habitats at 0.05 to 10 mL^{-1}, with most characterised as ice nucleation active. In addition, diatoms thought to be associated with ice nucleating bacteria in lake water were shown to produce ice at up to −3 °C [29]. Pietsch et al. [30] investigated the aerosolisation of two strains of *Pseudomonas* in a Collison nebuliser; air was injected at high velocity through the nebulizer's orifice which broke the cell suspension apart into small droplets. More recently, two studies have measured INPs in river surface waters. Moffett [31] surveyed the river Gwuan from its upper reaches to several kilometres out to sea for total and heat sensitive INP concentrations, while Larsen et al. [32] measured INPs weekly at one position in the Rhine, in Basel, Switzerland, over 16 months. Baseline Rhine river concentrations were <100 INPs active at −8 °C mL^{-1}, but this increased to around 4000 INPs mL^{-1} at −8 °C during transitory peak flows following intense rainfall, likely associated with the flushing of INPs from plant surfaces, litter, and soil. Most INPs were small (<0.22 μm).

The work documented here is the first analysis of INPs in a major river system. We demonstrate that the river waters contained high levels of biological INPs and suggest that their potential influence on cold cloud ice formation and precipitation requires investigation.

2. Materials and Methods

A single surface water sample was taken approximately every 150–250 km along the river systems (Figure 1). To sample river surface water, a sterile 15 mL centrifuge tube (Falcon, VWR, Lutterworth, UK) was taped to a 4 m length of plastic pipe. The assembly was then lowered into the water as vertically as possible and the top ~1 cm of water allowed to flow into the tube. These samples were then immediately frozen on dry ice for a maximum of 48 h or kept cold on crushed ice for at most 6 h before analysis. A second bulk sample of approximately 450 mL was then taken in the same way into a clean 500 mL polypropylene bottle (VWR), and within 5 min the temperature and pH determined. The pH was determined using a portable meter checked every day with standard buffers. INPs were counted using a thermal cycler, (PTC-200, MJ Research, Watford, UK) as described in Hill et al. [33], except in this case the thermal cycler had been modified to enable cooling to 30 °C below ambient. INP determinations were performed using 16–24 aliquots of 80–100 μL in a single-use 96-well polypropylene plate (Starlab, Milton Keynes, UK). The temperature was reduced in increments of 1 °C with a transition time of 5–10 s. Samples were then held for 2 min at each temperature before assessing the number of frozen wells visually. Conversion to INP mL^{-1} water at each temperature was estimated using the formula $-\ln(f)/V$, where f is the proportion of droplets not frozen and V is the volume of each aliquot [34]. The majority of samples were also heated in the modified thermal cycler to 95 °C for 20 min. Before reanalysis to determine the fraction of INPs which were likely to be biological, the trays were briefly centrifuged to remove small bubbles (these can obscure the appearance of freezing). Three pooled samples and one individual sample were also filtered through 0.22 μm-pore-diameter syringe filters (PES, VWR) to gauge the size of the INPs. The syringe filters contributed no INPs to the samples. Deionised water passed through a 20 nm filter served as controls in each determination (0.6% of control wells froze during these experiments).

The results for 49 samples are presented. The majority were sampled in a two-week period in June 2015 and the remainder in a two-week period in September 2012. One site (Lewellen, NE, USA) was sampled on both occasions.

3. Results

Sampling locations and numbers of INPs active at −10 °C or warmer are shown in Figure 1, whereas details of sampling location, water chemistry, and INP concentrations at −7 °C and −10 °C, as well as the % heat labile at −10 °C, are shown in Table 1. Water temperatures varied from 11.0 to 28.6 °C, while pH ranged from 6.8 to 9.0.

Figure 1. Sampling locations and ice nucleating particle concentrations (per millilitre active at −10 °C).

Ice spectra (the number of INPs per mL plotted against temperature) are shown for both untreated and selected heat treated (95 °C) samples separately for the Mississippi, Missouri, and Sweetwater/Platte Rivers in Figure 2 (complete results for heat treated samples are shown in Figure S1). The most efficient INPs initiated freezing at between −4 and −6 °C, while numbers active at −7 and −10 °C or warmer ranged from 36 to 12,000 mL^{-1} and 87 to 47,000 mL^{-1} river water, respectively (Table 1). In precipitation, the corresponding values mostly fall within the range 0.1 to 50 mL^{-1} [1]. Hence, direct inputs to rivers from precipitation (apart from that which intersects foliage) is a minor source. Heating reduced INP concentrations, typically to the limit of testing at −13 to −15 °C. At −10 °C or warmer, 66 to >99% of INPs were heat labile, and therefore likely to be biological [13] (Table 1).

Three samples from the 2012 campaign were filtered using 0.22 μm disposable filters. As shown in Table 2, the proportion of INPs in the filtrate was variable. Between 24 and >99% of INPs passed through the filters, suggesting that a significant proportion were not intact cells.

Table 1. Details of sampling locations and ice nucleating particle concentrations. SAREC = Sustainable Agriculture Research and Extension Centre.

River	Location	Date	Lat.	Long.	Water Temp. (°C)	pH	Onset of Freezing (°C)	INPs Active at −7 °C (mL^{-1})	INPs Active at −10 °C (mL^{-1})	INPs Heat Labile at −10 °C (%)
Sweetwater	Sweetwater Camp Site	8/15/2012	42.564837	−109.061902	11.0	6.8	−5	43	690	81
Sweetwater	Sweetwater Bridge	8/15/2012	42.541721	−108.18018	17.0	7.7	−6	180	1600	99
Sweetwater	Ore Road	8/15/2012	42.524983	−107.798983	21.0	8.0	−6	130	1200	66
Sweetwater	Mormon Bridge	8/14/2012	42.442715	−107.221725	-	-	−5	420	2100	78
North Platte	Miles Landing	8/16/2012	42.638502	−106.619033	17.0	7.3	−6	>69	130	67
North Platte	Bessemer Bend Rd	8/16/2012	42.771948	−106.530563	17.0	7.7	−5	>69	87	89
North Platte	Pacificorp, Casper	8/16/2012	42.865464	−106.297231	19.0	8.4	−4	87	540	76
North Platte	Glendo Dam	8/17/2012	42.478084	−104.951656	17.0	7.3	−4	370	1100	66
North Platte	Guernsey	8/17/2012	42.259881	−104.74157	20.0	8.6	−5	370	1500	75
North Platte	SAREC, Lingle	8/23/2012	42.125186	−104.378219	19.0	7.6	−5	340	1400	87
North Platte	Chimney Rock	8/24/2012	41.729665	−103.324214	18.0	7.5	−5	410	1800	95
North Platte	Lewellen	8/24/2012	41.316284	−102.126333	21.0	7.5	−5	130	980	91
North Platte	Lewellen	6/14/2015	41.316284	−102.126333	21.5	8.0	−4	1500	2600	69
Platte	Cozad	6/14/2015	40.837433	−99.98696	22.5	8.0	−5	1500	4700	99
Platte	Hastings	6/14/2015	40.77126	−98.492202	24.0	8.1	−5	1500	1700	94
Platte	North Bend	6/14/2015	41.452678	−96.776912	24.5	8.3	−5	1200	4700	97
Platte	La Platte	6/15/2015	41.058924	−95.928276	-	-	−5	4700	15,000	88
Missouri	Three Forks/Headwaters St. Pk.	6/28/2015	45.926835	−111.50578	22.1	8.6	−6	47	590	-
Missouri	Wolf Creek Br./Holter Dam	6/27/2015	47.020074	−112.011922	17.3	9.0	−6	36	170	-
Missouri	Old Fort Benton	6/27/2015	47.820359	−110.663314	24.3	8.9	−4	350	590	-
Missouri	James Kipp Recreation Area	6/27/2015	47.627895	−108.697475	27.5	8.8	−5	81	590	-
Missouri	Fort Peck	6/26/2015	48.039842	−106.290658	12.9	8.3	−5	870	1500	-
Missouri	Williston	6/26/2015	48.110958	−103.717126	21.5	8.1	−4	2600	10,000	-
Missouri	Washburn	6/26/2015	47.288804	−101.042415	11.0	8.4	−5	470	2100	-
Missouri	Mobridge	6/25/2015	45.512826	−100.382602	24.2	7.3	−5	170	360	-
Missouri	Bad River near Fort Pierre	6/25/2015	44.353082	−100.369376	23.2	7.8	−5	17,000	59,000	-
Missouri	Chamberlain	6/25/2015	43.815016	−99.326346	21.3	8.5	−5	170	470	-
Missouri	Blair	6/14/2015	41.548381	−96.097105	22.3	8.4	−4	1500	3000	90
Missouri	Plattsmouth	6/15/2015	41.000821	−95.866735	23.2	7.9	−4	3500	12,000	95
Missouri	Rulo	6/15/2015	40.049925	−95.421274	23.0	7.9	−5	4700	15,000	93
Missouri	Leavenworth	6/15/2015	39.330756	−94.910311	23.5	7.7	−4	7200	26,000	95
Missouri	Missouri City	6/15/2015	39.236795	−94.29664	22.7	7.7	−4	12,000	47,000	95
Missouri	Mokane	6/17/2015	38.670188	−91.874438	20.8	7.9	−5	1000	3600	90
Mississippi	Bemidji	6/23/2015	47.483244	−94.727823	20.3	8.0	−6	1000	1700	99
Mississippi	Brainerd	6/23/2015	46.34847	−94.20721	22.7	7.9	−5	590	5900	99
Mississippi	Monticello	6/22/2015	45.294468	−93.757178	25.2	8.2	−5	170	1700	97
Mississippi	Red Wing	6/22/2015	44.562502	−92.461088	23.9	8.4	−5	1500	3600	96
Mississippi	Pettibone Pk, La Crosse	6/22/2015	43.812197	−91.260732	24.7	8.4	−6	290	1700	95
Mississippi	Dubuque	6/22/2015	42.495042	−90.65702	23.2	8.1	−6	470	2100	93
Mississippi	Muscatine	6/21/2015	41.419779	−91.043757	24.4	7.9	−5	870	3500	93
Mississippi	Canton	6/21/2015	40.139674	−91.515404	23.9	8.0	−5	1700	4700	90

Table 1. *Cont.*

River	Location	Date	Lat.	Long.	Water Temp. (°C)	pH	Onset of Freezing (°C)	INPs Active at $-7\,°C$ (mL^{-1})	INPs Active at $-10\,°C$ (mL^{-1})	INPs Heat Labile at $-10\,°C$ (%)
Mississippi	Dam 25, Winfield	6/21/2015	39.0054	−90.691467	24.0	8.0	−5	2600	8700	99
Mississippi	Herculaneum	6/17/2015	38.258919	−90.376007	22.8	7.7	−4	2600	12,000	99
Mississippi	Cape Girardeau	6/17/2015	37.31516	−89.512691	25.8	7.7	−5	2600	12,000	97
Mississippi	Caruthersville	6/17/2015	36.232633	−89.702174	28.0	8.6	−5	210	720	76
Mississippi	Mud Island, Memphis	6/18/2015	35.18217	−90.057939	28.6	7.4	−5	150	470	92
Mississippi	Rosedale	6/18/2015	33.827979	−91.053996	27.1	7.8	−6	87	170	90
Mississippi	Mayersville	6/18/2015	32.917046	−91.061286	26.8	7.7	−5	1700	8700	96
Mississippi	Natchez	6/18/2015	31.558579	−91.413607	26.6	7.7	−5	1500	4700	93

Table 2. Ice nucleating particles active at −7 and −10 °C in 0.22 µm filtrate. Up to three contiguous samples were combined for each test.

Samples	INPs < 0.22 µm Active at −7 °C (%)	INPs < 0.22 µm Active at −10 °C (%)
Sweetwater (Campsite, Bridge, Ore Road)	100	97
North Platte (Glendo Dam, Guernsey)	31	32
North Platte (SAREC, Lingle)	44	24
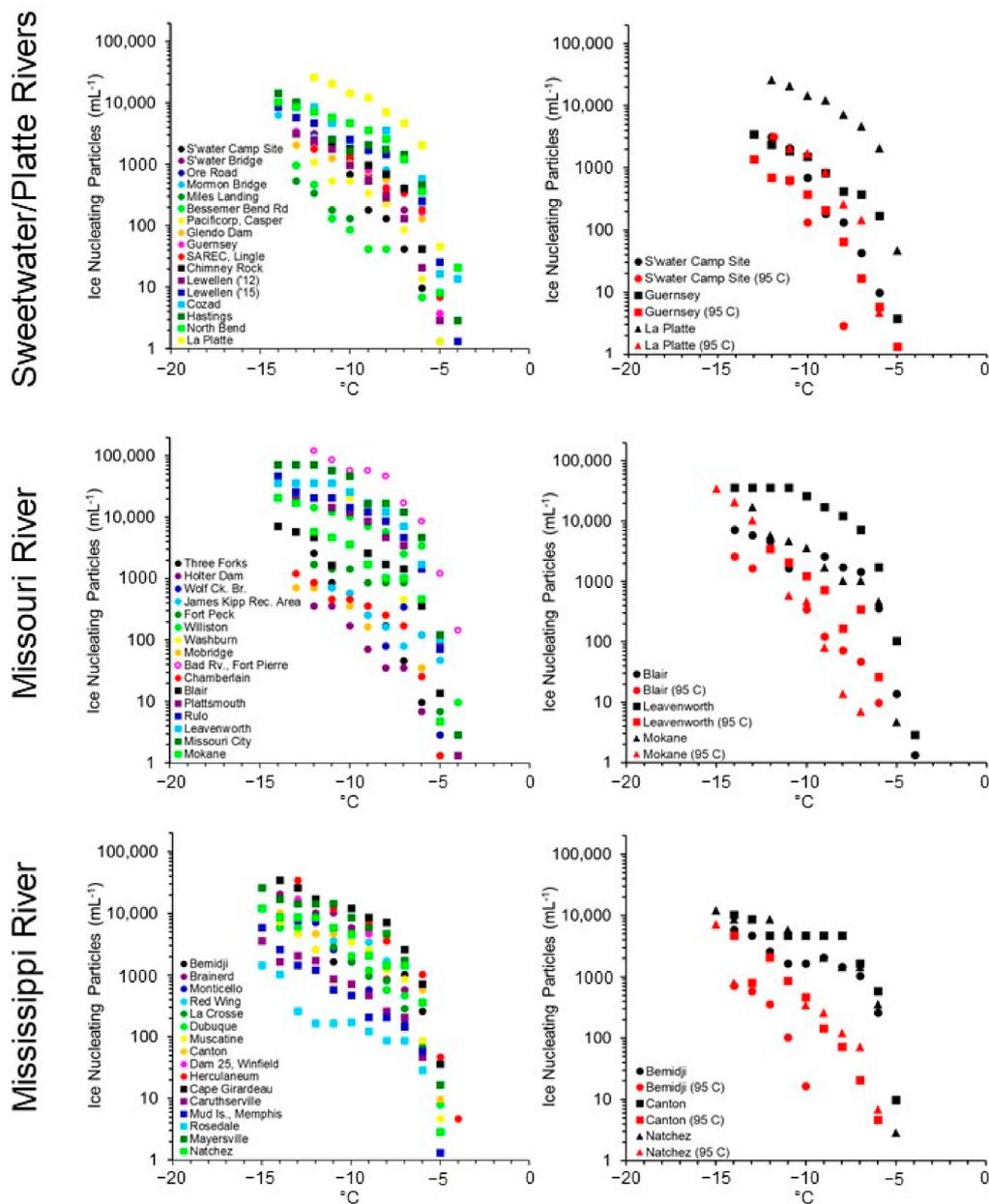
Figure 2. Full spectra of ice nucleating particle concentrations in the Sweetwater/Platte, Missouri and Mississippi Rivers. (**Left**) column figures show INP spectra in untreated water, while the (**right**) column figures give selected results (upper, middle, and lower reaches of each river system) for untreated and heat-treated (95 °C for 20 min) samples.

4. Discussion

In this study, the concentrations of INPs in surface river water were much greater than that typically found in marine systems: median values at $-10\ °C$ were around 2000 times that of seawater, which tends to be $\leq 1\ mL^{-1}$ at $-10\ °C$ [25,31] with occasionally higher transitory concentrations, probably associated with algal blooms [25]. The number of INPs was also highly variable, reflecting the relative contributions of a range of sources (Figure S2). The vast majority of INPs (66–99%, median 93%) were heat labile, suggesting a biological component. Indeed, the susceptibility of INPs to being denatured by 95 °C generally increased with their concentration in the sample (Figure 3). It can also be seen that the warmer-temperature-active INPs are more heat sensitive and that the effect is lost around $-12\ °C$ (Figure 2 and Figure S1); presumably, at colder temperatures INPs are a mix of heat-resistant organic and mineral INPs [12]. The size observations agree with previous work on freshwater INPs in Wales [31], marine INPs [17,20], precipitation [35–37], and recent studies showing that ice nucleating fungi and pollen release copious numbers of small (e.g., <15 nm) cell-free INPs [38–40].
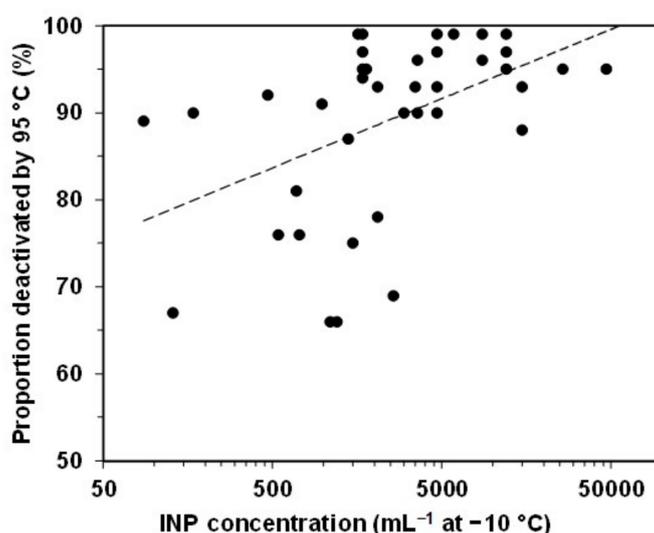
Figure 3. Relationship between INP concentration and heat susceptibility ($R^2 = 0.23$).

Figure 1 highlights some of the geographic variation in the distribution and concentration of INPs. One point of interest is the confluence of the Mississippi River with its largest tributary, the Ohio. The two rivers are very different, with the turbid Mississippi living up to its moniker "The Big Muddy". There was a marked decrease (>15-fold) in INP concentration below their junction (Figure 1). On the day of sampling, the discharge of the Mississippi upstream of the junction was 14,450 $m^3\ s^{-1}$ with a suspended sediments loading of 1110 mg L^{-1} (monitored at Thebes; USGS National Water Information System, Site number 07022000), whereas that of the Ohio was only 4100 $m^3\ s^{-1}$ (at Olmsted, USGS 03612600, no suspended sediments or turbidity data available at this or nearby stations). Hence, simple dilution of the Mississippi's INPs by a large influx of "cleaner" water from the Ohio did not cause the decrease. It, therefore, may have resulted from microbiological decomposition of organic INPs or from sedimentation due to a change in river chemistry. For example, at the time of sampling the pH of the Mississippi was 7.7–8.0, whereas in the Ohio it was 6.9. In non-alkaline water, divalent cations are more soluble and hence available to form flocs. An injection of Mg^{2+} and Ca^{2+} ions by the Ohio may have promoted flocculation and rapid settling of INPs downstream of the confluence.

By contrast, the large jump in INP concentration in the Missouri, from 1500 to 10,000, as it crossed from Montana into North Dakota (Figure 1), was likely due to large inflows of INP-rich water from the Yellowstone River just upstream of the Williston sampling point. During the afternoon of sampling, the Missouri's discharge was only 250 $m^3\ s^{-1}$ (measured near Culbertson; USGS 06185500,

no suspended sediments or turbidity data available) whereas the Yellowstone was three times higher (830 m^3 s^{-1} and 155 mg L^{-1} suspended sediments, measured near Sidney, USGS 06329500). Interestingly, and as was also seen below the Mississippi/Ohio junction, INP concentrations then decreased as the Missouri flowed southeast through the Dakotas.

Although based on a limited number of observations, the level of sediment (a combination of upstream land use and rate of flow) in rivers may be directly related to the number of INPs. For example, the Bad River at Fort Pierre was sampled at its junction with the Missouri. This small tributary of the Missouri is noted for its very high silt levels, which was evident at the time of sampling. Accordingly, it had the greatest number of INPs of any sample: 17,000 mL^{-1} at −7 °C and 59,000 mL^{-1} at −10 °C. Post precipitation run-off would produce a pulse of increased sediment inputs, while washing of foliage by rain would contribute additional INPs. In addition, the swollen river would wash additional areas of vegetation. The increased flow rate may also erode biofilms on river bed stones and pebbles, and stir up sediments on the river bed.

Freshwater could be a significant source of regional atmospheric INPs. Indeed, freshwater bacteria have even been found to dominate the bacterial microbiome of aerosols [41,42]. The same emission mechanism that operates in seawater—bubble bursting—would also facilitate freshwater INPs becoming airborne. Bubble bursting has been assessed to cover between 3–4% of the ocean's surface at any one time [43]. This is likely to be greater for large freshwater bodies because the waves are smaller and occur with much greater frequency, and so break and foam more frequently [44]. In addition, film jet bubble bursting may be more extensive in freshwaters compared to marine due to the increased dissolved organic carbon content. This may be less significant in rivers during low flow rates, as demonstrated for the Rhine in Switzerland [32]. Using single particle aerosol time-of-flight mass spectrometry of aerosols generated from Lake Michigan water, Axson et al. [45] found a mass spectral fingerprint dominated by calcium carbonate but also with organic nitrogen markers consistent with the presence of organic material.

This initial study requires extension and refinement. Samples should be taken at different seasons, depths, and distances from the bank to build up a more detailed picture of freshwater INP dynamics and to assess the rate at which freshwater INPs become airborne and the factors, such as the influence of the surface microlayer, controlling it. Initial results from the Maumee River suggest that freshwater INPs do indeed become aerosolised [46]. Other rivers and, especially, large freshwater bodies such as lakes, reservoirs, glaciers, swamps, peat bogs, and other wetlands should also be analysed to see if these initial findings are broadly representative. For example, most samples in this study were taken from regions where agriculture predominates; other freshwater bodies may not reflect this anthropo-ecological context. As regards the nature of the INPs, at this stage we can only presume that they are macromolecules such as proteins; work is ongoing to characterize them. However, a testable hypothesis resulting from this initial work is that freshwater bodies are a significant repository of biological INPs, which are likely to vary seasonally and impact regionally on cloud ice formation, precipitation, and climate.

Conflicts of Interest: The authors declare no conflict of interest.

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