



**QUEEN'S  
UNIVERSITY  
BELFAST**

## Water activity in Venus's uninhabitable clouds and other planetary atmospheres

Hallsworth, J. E., Koop, T., Dallas, T. D., Zorzano, M. P., Burkhardt, J., Golyshina, O. V., Martín-Torres, J., Dymond, M. K., Ball, P., & McKay, C. P. (2021). Water activity in Venus's uninhabitable clouds and other planetary atmospheres. *Nature Astronomy*, 5(7), 665-675. <https://doi.org/10.1038/s41550-021-01391-3>

**Published in:**  
Nature Astronomy

**Document Version:**  
Peer reviewed version

**Queen's University Belfast - Research Portal:**  
[Link to publication record in Queen's University Belfast Research Portal](#)

**Publisher rights**  
Copyright 2021 Nature. This work is made available online in accordance with the publisher's policies. Please refer to any applicable terms of use of the publisher.

**General rights**  
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

**Take down policy**  
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact [openaccess@qub.ac.uk](mailto:openaccess@qub.ac.uk).

**Open Access**  
This research has been made openly available by Queen's academics and its Open Research team. We would love to hear how access to this research benefits you. – Share your feedback with us: <http://go.qub.ac.uk/oa-feedback>

# Water activity in Venus' uninhabitable clouds and other planetary atmospheres

John E. Hallsworth<sup>1,\*</sup>, Thomas Koop<sup>2</sup>, Tiffany D. Dallas<sup>1</sup>, María-Paz Zorzano<sup>3,4</sup>, Juergen Burkhardt<sup>5</sup>, Olga V. Golyshina<sup>6</sup>, Javier Martín-Torres<sup>4,7</sup>, Marcus K. Dymond<sup>8</sup>, Philip Ball<sup>9</sup>, and Christopher P. McKay<sup>10</sup>

<sup>1</sup>*Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, 19 Chlorine Gardens, Belfast, BT9 5DL, Northern Ireland, UK.*

<sup>2</sup>*Faculty of Chemistry, Bielefeld University, 33615 Bielefeld, Germany.*

<sup>3</sup>*Centro de Astrobiología (CSIC-INTA), 28850 Torrejón de Ardoz, Madrid, Spain.*

<sup>4</sup>*Department of Planetary Sciences, School of Geosciences, Meston Building, King's College, University of Aberdeen, Aberdeen, AB24 3UE, Scotland, UK.*

<sup>5</sup>*University of Bonn, Institute of Crop Science and Resource Conservation, Plant Nutrition Group; Karlrobert-Kreiten-Str. 13, 53129 Bonn, Germany.*

<sup>6</sup>*School of Natural Sciences and Centre for Environmental Biotechnology, Bangor University, Gwynedd, LL57 2UW, Wales, UK.*

<sup>7</sup>*Instituto Andaluz de Ciencias de la Tierra (UGR-CSIC), 18100 Armilla, Granada, Spain.*

<sup>8</sup>*Division of Chemistry, School of Pharmacy and Biomolecular Sciences, University of Brighton, Lewes Road, Brighton BN2 4GL, England, UK.*

<sup>9</sup>*Hillcourt Road, London SE22 0PE, England, UK.*

<sup>10</sup>*Space Science Division, NASA Ames Research Center, Moffett Field, California, 94035, USA.*

\*e-mail: j.hallsworth@qub.ac.uk

**The recent suggestion of phosphine in Venus' atmosphere has regenerated interest in the idea of life in clouds. However, such analyses usually neglect the role of water activity, which is a measure of the relative availability of water, in habitability. Here, we compute the water activity within the clouds of Venus and other Solar-System planets from observations of temperature and water-vapour abundance. We find water-activity values of sulphuric acid droplets, which constitute the bulk of Venus' clouds, of  $\leq 0.004$ , two orders of magnitude below the 0.585 limit for known extremophiles. Considering other planets, ice formation on Mars imposes a water activity  $\leq 0.537$ , slightly below the habitable range, whereas conditions are biologically permissive ( $> 0.585$ ) at Jupiter's clouds (although other factors such as their composition may play a role in limiting their habitability). By way of comparison, the Earth's troposphere conditions are, in general, biologically permissive, whereas the atmosphere becomes too dry for active life above the middle stratosphere. The approach used in the current study can also be applied to extrasolar planets.**

40 There is currently a surge of interest in terrestrial aerobiology, and we now know that airborne  
 41 microorganisms can be metabolically active<sup>1,2</sup>. As long as temperatures are biologically  
 42 permissive, the abundance of liquid water within the Earth's atmosphere favours physiological  
 43 activity. The thermodynamic parameter water activity, the ratio between the water vapour  
 44 pressures of the solution and of pure water under the same conditions, is used to quantify the  
 45 availability of water. This parameter applies to all solutions and phases; liquid, solid, and gas.  
 46 For planetary-atmosphere applications, water activity is equivalent to relative humidity. The  
 47 maximum possible value of equilibrium relative humidity is arbitrarily designated as 100%,  
 48 whereas the maximum possible water activity is attributed a value of 1, for a given temperature-  
 49 and pressure combination. Water activity acts as a potent determinant of functionality for  
 50 microbial cells<sup>3</sup>, so is also a key determinant of habitability<sup>4</sup>.

51 This raises the question of possible life in atmospheres beyond Earth. The atmospheres  
 52 of other planetary bodies exhibit various combinations of temperature, pressure, and relative  
 53 humidity - parameters that can be obtained through either calculations or direct measurements.  
 54 Even if temperatures within the atmospheres of other planets permit the formation of liquid  
 55 water-containing droplets and may seem permissive for life as we know it, we must still ascertain  
 56 the water activity of these droplets. This parameter is not only influenced by temperature and  
 57 pressure, but also by thermodynamic effects of ice and/or the presence of any solutes or co-  
 58 solvent(s).

59 The recent suggestions of biogenic substances such as phosphine in the Venusian  
 60 clouds<sup>5,6</sup> is continuing a history of speculation about life on Venus<sup>7-9</sup>. Venus' surface is  
 61 considered too hot for organic life-forms but the lower cloud layer, at an altitude of ~40 to 70 km,  
 62 has a temperature range that makes it potentially habitable based on our knowledge of  
 63 terrestrial-type life. Earlier studies suggested the lack of liquid water, or at least the low  
 64 availability of water, as a potential barrier to life<sup>10,11</sup>. Although several recent analyses have  
 65 queried whether adequate water is available, they also propose active cellular metabolism in the  
 66 sulphuric acid-rich droplets of the Venusian atmosphere<sup>5, 12, 13</sup>.

67 A thorough assessment of biophysical limits-for-life on Earth was carried out in the  
 68 context of planetary protection by a 2013-2014 committee of the Mars Exploration Program  
 69 Analysis Group (MEPAG) of NASA that aimed to identify 'Special Regions' of Mars<sup>4</sup>; i.e., places  
 70 that are biologically permissive for active terrestrial-type life. The MEPAG report identified that  
 71 some metabolic processes occur down to -40°C, i.e., below the recognised (-18°C) limit for cell  
 72 division, and stated that microbial metabolism and cell division had been documented only down  
 73 to a water activity of 0.605 (the limit for cell division of the fungal xerophile *Xeromyces bisporus*).  
 74 From a more-recent study, we now know that metabolism, differentiation, and cell division can  
 75 occur down to a water activity of 0.585 (from a study of the fungal halophile/xerophile *Aspergillus*  
 76 *penicilliioides*)<sup>14</sup>. We also know that microbes can remain dormant at water-activity values below

77 their window for biotic activity, and then resume metabolism when water activity increases<sup>15</sup>. At  
78 the upper end of the water-activity scale, many microbes are active at a value of 1<sup>ref. 16</sup>; at high  
79 temperatures microbial growth has been observed up to about 121°C<sup>4</sup>, but circumstantial  
80 evidence hints at possible metabolism close to 130°C.

81 Here, in the light of this knowledge, including recent revisions on our understanding of  
82 acidity- and water-activity limits for terrestrial microbes (see also below), we focus on Venus as a  
83 case study to quantify the water activity of clouds and determine whether terrestrial-type life is  
84 feasible there. However, the possibility of life in clouds can extend beyond Venus, so we also  
85 consider whether clouds on Jupiter and Mars have temperature- and water-activity values  
86 consistent with habitability.

87  
88 **Water activity and uninhabitability of Venus' clouds.** There is no *a priori* reason to suppose  
89 that putative Venusian life would have the same biochemical basis as that on Earth. In the ab-  
90 sence of any concrete proposal for an alternative biochemistry, however, several studies have  
91 considered whether living systems comparable to those on Earth might find viable niches on Ve-  
92 nus, suggesting that this might be possible within the droplets of sulphuric acid clouds<sup>5, 12,13</sup>.

93 Concrete information about biophysical limits for cellular function comes primarily from la-  
94 boratory-based studies of terrestrial extremophiles (see Methods subsections *Acidity- and water-*  
95 *activity limits-for-active-life on Earth* and *Determination of habitability for Venus' acid clouds*;  
96 Supplementary 'Text on biophysical limits of terrestrial microbes'). These data indicate that mi-  
97 crobial growth and metabolism cannot occur anywhere near the chemical conditions relevant for  
98 Venusian clouds, as revealed below (in this section). Much of the discussion of potential life in  
99 the Venus atmosphere, both in the light of recent work<sup>5</sup> and previous studies, focuses on the  
100 extreme acidity of sulphuric acid clouds<sup>12,13,17</sup>. This is not in itself an obvious obstacle because  
101 we know that some extreme acidophiles are capable of metabolism close to, and even below,  
102 pH 0 (see references within<sup>13</sup>). Indeed, it is sometimes assumed that the most-acidophilic mi-  
103 crobe can even grow in concentrated sulphuric acid. However, the record holder, the archaeon  
104 *Picrophilus torridus*, grows down to a pH of -0.06 (at 60°C)<sup>18</sup> which is equivalent to only about  
105 11.5% (w/w) sulphuric acid<sup>19</sup>.

106 Given that a permissive water activity is a prerequisite for active metabolism of terrestrial  
107 life-forms, we considered the water activity within Venus' clouds. We determined how sulphuric  
108 acid modifies water-vapour pressure of a liquid phase by converting water-vapour pressure data  
109 for H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O mixtures<sup>20</sup> to water-activity values (Figure 1; data are provided in Supplementary  
110 Table 1). We observed a strong reduction of water activity even at modest sulphuric acid  
111 concentrations. We employed two independent thermodynamic models of sulphuric acid-water  
112 mixtures. The first model was from a study of Gmitro and Vermeulen<sup>20</sup> and is particularly well-  
113 suited for medium-to-high concentrations of (and up to pure) sulphuric acid over the entire  
114 temperature range of relevance to the current study. The second, the Extended Aerosol

Inorganics Model (E-AIM) by Clegg *et al.*<sup>19</sup> describes water activity and ion activities in multicomponent solutions, including pH, and is applicable to sulphuric acid solutions of between 0 and ~80% (w/w) at temperatures from <-73°C to +55°C. For Figure 1, water-activity values at temperatures that are pertinent to life between -40 and 130°C were determined from the data presented in water-vapour pressure tables, calculated by Gmitro and Vermeulen from their model<sup>20,21</sup>, at sulphuric acid concentrations between 10 and 100% (w/w); intermediate values were then obtained by interpolation (see Supplementary Table 1 and Methods).

Concentrations of 35.0, 37.5, 40.1 and 42.3% (w/w) sulphuric acid reduce water activity to the currently recognised (0.585) limit-for-life at -40, 0, 50 and 100°C (233, 273, 323, and 373 K), respectively (black iso-line in Figure 1). These sulphuric acid concentrations are consistent with the E-AIM<sup>19</sup> for temperatures at -40, 0 and 50°C (35.6, 37.7 and 40.3) and those published elsewhere for temperatures between 0 and 75°C (273 and 348 K)<sup>22</sup>, see Supplementary Figure 1 as well as Supplementary Tables 2a and 2b for comparison.

Here, we derive the water-activity levels in Venus' clouds from direct observations and then determine the sulphuric acid concentration that corresponds to this water activity according to the solution chemistry. The relative humidity of the atmosphere can be calculated directly using observations of temperature, pressure, and water-vapour mixing ratio<sup>23-26</sup>. At the altitudes pertinent to biology (40 to 70 km; i.e., about +130 to -40°C), the relative humidity of the atmosphere varies yet remains less than 0.40% throughout this range. Because of the small droplet size, the water of the droplets and the ambient water-vapour are assumed to be in equilibrium<sup>27,28</sup> (see also Methods), hence, these relative humidity values correspond to water activities in the droplets below 0.004 (i.e., from 0.00003 to 0.0037; Table 1; Figure 2). Using data from Gmitro and Vermeulen<sup>20,21</sup> (see Methods), we observe that these water-activity values correspond to sulphuric acid concentrations of 77.8 to 99.2% (w/w) throughout the putative habitable zone, as indicated by the grey circles in Figure 1 (see also Table 1). These concentrations are consistent with those of Clegg *et al.*<sup>19</sup> in the temperature range -40 to 25°C (even though they lie beyond the stated validity range of the E-AIM). Our results for water activity are consistent with published observations and published model calculations of acid content in Venus' clouds<sup>29-31</sup>. Given that Earth's entire functional biosphere spans only about 0.415 water-activity units, from 1 to 0.585<sup>ref. 32</sup>, the thermodynamic distance between the 0.585 water-activity limit and the water activity of Venus' cloud droplets seems unbridgeable (Figure 1). In other words, there is an enormous distance on the water-activity scale between the limits for metabolism of terrestrial extremophiles and the conditions of the Venus cloud layer (Figure 3). Furthermore, terrestrial life cannot survive the extreme acid concentrations equivalent to those found in the Venus clouds (see below; Figure 3).

For cloud droplets to be habitable, their water activity would have to be strongly out of equilibrium, meaning that the water activity is not determined by ambient relative humidity. Indeed, their water activity would have to be enhanced by a factor of about 150 with respect to

the ambient water vapour. In general, authors of different studies (ourselves included) agree that the droplets making up Venus' clouds are in equilibrium with the atmosphere; both data and theoretical evidence support this view (Supplementary 'Text relating to equilibration of droplets')<sup>5,29</sup>.

Note that even at temperatures below 0°C, no ice can form in the cloud layer at such low water-activity values. In the binary-phase diagram of sulphuric acid and water<sup>33</sup>, several crystalline sulphuric acid hydrates are stable at lower temperatures but, at the high acid-concentrations considered here (of > 78%, w/w), in principle only the sulphuric acid monohydrate or pure crystalline sulphuric acid can form. However, laboratory experiments showed that neither of these phases crystallises readily, even in bulk samples, and that they instead form metastable-solution droplets<sup>33</sup>. Furthermore, observations in Earth's stratospheric aerosol layer support this notion<sup>30</sup>.

Seager *et al.*<sup>13</sup> speculate that Venusian microbes would have adaptations to capture and retain water, but we have yet to identify any terrestrial microbe able to obtain and accumulate water from the vapour phase at <0.40% relative humidity<sup>4</sup>. Furthermore, we believe that under the hostile conditions in the Venus atmosphere any cells would likely perish (even dormant cells); see subsection *Acidity- and water-activity limits-for-active-life on Earth* below. The water-activity limits-for-active life are determined by thermodynamics and the need for water as the biophilic solvent for complex macromolecules<sup>34</sup>. At low water-activity, microbial cells adapt to retain their functionality by changing the composition of the plasma membrane; accumulating stress metabolites<sup>35</sup>; preferentially accumulating chaotropic substances at low temperatures to retain flexibility of their macromolecular systems<sup>36</sup>; and in other ways. Whereas such adaptations mitigate against stresses induced by low water-activity, osmotic stress, and other biophysical activities of solutes, this mitigation only extends the windows for cellular functionality within finite limits and cannot circumvent the need for a biologically permissive water activity<sup>36-38</sup>.

In their analysis of cloud habitability, Seager *et al.*<sup>13</sup> state that cells can be destroyed due to the chemical modifications that can occur to metabolites and cellular macromolecules (these are detailed in our Figure 3). This is consistent with studies showing that extremely acidic solutions rapidly kill the cells of many microbes at pH < 1<sup>ref. 39</sup>. However, we argue that the problems go far deeper. We can see that sulphuric acid, at concentrations thought to be relevant to Venusian cloud droplets, reduces water activity far below a level where it can function as a biophilic solvent for complex macromolecules (Table 1; Figures 1 and 3). Sulphuric acid dehydrates the cellular systems, removes water from biomacromolecules, reduces hydrophobic interactions, and damages plasma-membrane integrity (Figure 3). For both the polyextremophile *Acidihalo-bacter aeolianus* (see Methods subsection *Acidity- and water-activity limits-for-active-life on Earth*) and the thermoacidophile *P. torridus*, sulphuric acid tolerance limits for growth are an order of magnitude lower than those found in the clouds of Venus (Figure 3).

It is important to remember what the hydration shell of a protein actually does for func-

tionality. There is evidence that the surface of a typical protein (lysozyme, for example) must have at least 50% water coverage to be functional<sup>40</sup>. This is thought to correspond to 66% of the purely hydrophilic regions, and to coincide with a percolation threshold in two dimensions. Of course, one cannot assume that macromolecules in a Venusian organism would share the same features as those of terrestrial organisms, but the real point is what role this water coverage plays. The dynamics of proteins and their hydration spheres are closely coupled, and it is thought that fluctuations of the solvent (due to spontaneous rearrangements of the hydrogen-bonded network) are needed to ‘awaken’ those in the protein and give it the plasticity required for functionality<sup>41</sup>. For additional information, see Methods subsection *Determination of habitability for Venus’ acid clouds*; and Supplementary ‘Text for Figure 3’.

**Analyses for clouds of Jupiter, Mars, Earth, and exoplanets.** Determinations of water activity, as an important first step in assessing habitability of clouds, can also be made for Jupiter, Mars, Earth, and exoplanets. For Jupiter, there has been only one entry probe and it appears to have entered into an unusually dry region of the Jovian atmosphere. Furthermore, dry and wet regions are present in a complex pattern of local meteorology<sup>42</sup>. Here, we use the temperature-pressure profiles from this probe; the Galileo Atmospheric Entry Probe<sup>43</sup>. The water-vapour mixing ratio, i.e., the molar ratio of water gas to all other gases present, is not well constrained. The Galileo Atmospheric Entry Probe measured water-vapour mixing ratios of  $(4.7 \pm 1.5) \times 10^{-5}$  at 11–11.7 bar and  $(4.9 \pm 1.6) \times 10^{-4}$  at 17.6–20.9 bar<sup>44</sup>. These values are about 3 and 30%, respectively, of the water-vapour mixing ratios expected if water on Jupiter had the same relative abundance of oxygen and hydrogen as the Sun. Conversely, Li *et al.*<sup>45</sup> used the results from the Juno mission, which orbited Jupiter, to infer that the water-vapour mixing ratio in the range of approximately 0.7 to 30 bar is about 2.7 times the value expected for solar abundances. Although it is clear that the water-vapour mixing ratio is variable and the measurements uncertain, the value set by solar abundances deep in the Jovian atmosphere is plausible. This value is  $1.71 \times 10^{-3}$  ref. 46.

Higher in Jupiter’s atmosphere, the water-vapour mixing ratio is likely to be reduced due to the removal of water by condensation. However, in the region of interest between 0.1 and 20 bar the atmospheric temperature profile decreases smoothly with decreasing pressure so there is no temperature minimum to act as a cold trap<sup>43</sup>. Thus, a uniform mixing ratio of water vapour throughout this region is plausible (see for example ref. 46). If anything, this assumption will overestimate the water activity at lower pressures on Jupiter. Figure 4 shows water activity in the Jovian atmosphere as a function of temperature, from 50°C (at 4.6 bar) to 62°C (at 6 bar). Condensation occurs at about 10°C (at 5.5 bar) and becomes ice below 0°C (strictly, there is also an effect of pressure on water- and ice activity, but this is likely negligible given the other uncertainties). With further decreases in temperature, the water activity of ice decreases. In this analysis, we have neglected the effects of ammonia (NH<sub>3</sub>) or other atmospheric components on

the water activity of liquid water or on the freezing-point depression of the ice. These effects become more important near the ammonia clouds found at higher elevations (lower pressures and lower temperatures) that are not pertinent to the current analysis. The water activity is suitable for life ( $>0.585$ ) for temperatures between approximately  $10^{\circ}\text{C}$  and  $-40^{\circ}\text{C}$ . The atmosphere of Jupiter may, therefore, be more suitable for hosting terrestrial-type life than that of Venus (but whether it could serve as a suitable location for initiating life is another matter).

For Mars, even a casual inspection of the *in-situ* conditions reveals that the clouds are not biologically permissive due to the low temperatures that are not consistent with cellular function (there is also high ultra-violet radiation that can be lethal for atmospheric microbes<sup>47</sup>). Whereas clouds have been observed in the atmosphere of Mars, the temperatures are less than  $-73^{\circ}\text{C}$  ( $200\text{ K}$ ) regardless of altitude or location as determined by entry probes and global remote sensing<sup>48,49</sup>. Therefore, at best there are ice clouds, and microbial cells are not known to be able to access water at these extremely low temperatures. Furthermore, the water activity of ice at  $-73^{\circ}\text{C}$  is about  $0.537$ , and then drops sharply with decreasing temperature (computed from vapour-pressure formulae of liquid water and ice in <sup>ref. 50</sup>), so the water activity is also below the limit for active terrestrial-type life.

For Earth, typical water-mixing ratio profiles show that the upper stratosphere and mesosphere are too dry to be permissive for active life, while in the troposphere water activity is very variable spanning the entire range between  $0$  and  $1$ , depending upon location, season and daily weather (Figures 5 and 6; see Methods for details). Nearly all clouds in Earth's atmosphere are composed of either liquid water or water ice; the water activity of liquid water droplets is practically  $1$  (by definition) so is permissive for active life. The water activity of ice clouds, such as tropospheric cirrus clouds, depends upon temperature and is in theory biologically permissive down to  $-58^{\circ}\text{C}$  ( $215\text{ K}$ ). We note that temperatures below this  $-58^{\circ}\text{C}$  limit typically occur only in the upper troposphere, polar stratosphere and in the mesosphere. Our analysis suggests the vast majority of clouds in the troposphere are above the water-activity limit of  $0.585$  and, thus, consistent with active life.

Our approach of using temperature, pressure and water-vapour mixing ratio profiles to determine water activity can be applied to exoplanets. In principle, values for these three parameters can be calculated from transit measurements as an exoplanet moves in front of its star. Considering the problem in general, we can work out the case for an exoplanet atmosphere assuming that the water-vapour mixing ratio is set by the solar ratio of  $\text{O}/\text{H}$ . For example, Kreidberg *et al.*<sup>51</sup> found the water-vapour abundance of a Jupiter-sized exoplanet to be equal to the value for the Sun within the measurement uncertainties. Figure 7 shows a generalised water-activity analysis for exoplanets: the curve of pressure and temperature that corresponds to the water activity currently regarded as the minimum for active life ( $0.585$ ) (see also Supplementary 'Text for Figure 3'). Profiles to the right of this curve will have a lower water activity, while profiles to the left will have a higher water-activity. Ice clouds will have a lower water-activity set by their



temperature. For this reason, the lowest temperature considered is  $-59^{\circ}\text{C}$  because below that value, the water activity of ice is less than 0.585.

**Implications and Perspectives.** For a desiccated environment, whether a planetary atmosphere, surface or subsurface, the presence of liquid water does not necessarily indicate habitability. Temperature must be permissive for cellular integrity and function, energy sources and nutrients must be available and, critically, water activity must also be permissive for life. This is illustrated by our case study of Venus' clouds where viable microbe-water relations are a key prerequisite that – based on our knowledge of life on Earth – cannot be ignored. Indeed, it is only the two parameters of temperature and water activity that are considered determinants of habitability for the purposes of planetary protection<sup>4</sup>. It has been suggested that the droplets of Venus' clouds can act as a protective environment for microorganisms<sup>13</sup>, but we believe that cells could not retain their integrity and/or functionality there due to the low water-activity and biophysical and chemical effects of the highly concentrated sulphuric acid. Whereas we find the Greaves *et al.*<sup>5</sup> report of phosphine to be highly intriguing, other studies refute this finding, for example <sup>ref. 52,53,54</sup>. Based on the current study, we must imagine a qualitatively new type of organism to invoke a plausible story about life in the Venus atmosphere, at least for life as we know it.

Based on the findings of the current study (in relation to water-activity and temperature), the Jovian conditions make Jupiter's clouds currently the most-likely cloud formations in which life could exist in our Solar System apart from those of Earth. A similar analysis would be of interest for Saturn, Uranus, or Neptune, once entry-probe missions have been conducted. At present, we lack empirical data for suitable exoplanet atmospheres. However, we mapped out an approach whereby water-activity determinations can be made. It may be that such analyses form a key part of assessments in future to identify exoplanets based on determinations of their habitability. The James Webb Space Telescope (JWST) will be able to determine atmospheric profiles of temperature, pressure, and water abundance in exoplanet atmospheres<sup>55</sup>, and these will allow assessments of water activity in their atmospheres using our approach.

On Earth, life in the atmosphere has co-evolved with life in the oceans, on the surface, and in the subsurface. Terrestrial microorganisms are known to influence atmosphere composition, hydrological cycle, and meteorology. For example, microbes produce and consume various greenhouse gases; synthesise the stress metabolite dimethyl sulphide that volatilises and influences climate<sup>56</sup>; nucleate ice<sup>57</sup>; and drive cloud formation and precipitation. According to the findings of the current study, the Earth's troposphere is for the most part biologically permissive, the middle- and upper atmosphere become too dry for active life. Follow-on studies are also needed to consider the type of intimate relationship that can occur between the atmosphere's microbiome and other aspects of the planetary atmosphere, including climate and weather.

It should also be noted that on Earth (and to a lesser extent on Jupiter and possibly on Venus), atmospheric conditions can be dynamic. Therefore, the calculations presented here

should be considered as representative rather than fixed. This said, the temperature, pressure, and composition can be determined by direct measurements of planetary atmospheres (from probes or by remote-sensing methods) and these data provide a way to assess water activity. This methodology has planetary protection implications in relation to the potential designation of planetary atmospheres as 'Special Regions'. We believe that the quantitative tools developed here can also be used to determine the water activity in exoplanet atmospheres thereby narrowing the search for life within our Solar System and beyond.

### Data availability

Authors confirm that all relevant data are included in the paper and/ or its supplementary information files. Source data are provided with this paper (see Supporting Information Files; 'Source Data file for Figure 1', 'Source Data file for Figure 2', 'Source Data file for Figure 4', 'Source Data file for Figure 5', 'Source Data file for Figure 6a', and 'Source Data file for Figure 7').

### References

1. DeLeon-Rodriguez, N. *et al.* Microbiome of the upper troposphere. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 2575–2580 (2013).
2. Kabir, E. *et al.* Recent advances in monitoring, sampling, and sensing techniques for bioaerosols in the atmosphere. *ACS Sens.* **5**, 1254–1267 (2020).
3. Stevenson, A. *et al.* Is there a common water-activity limit for the three domains of life? *ISME J.* **9**, 1333–1351 (2015).
4. Rummel, J. D. *et al.* A new analysis of Mars "Special Regions": Finding of the Second MEPAG Special Regions Science Analysis Group (SR-SAG2). *Astrobiology* **14**, 887–968 (2014).
5. Greaves, J. S. *et al.* Phosphine gas in the cloud decks of Venus. *Nat. Astron.* <https://doi.org/10.1038/s41550-020-1174-4> (In press, 2020).
6. Mogul, R., Limaye, S. S., Way, M. J. & Cordova, J. A. Venus' mass spectra show signs of disequilibria in the middle clouds. *Geophys. Res. Lett.* **48**, e2020GL091327 (2021).
7. Morowitz, H. & Sagan, C. Life in the clouds of Venus? *Nature* **215**, 1259–1260 (1967).
8. Limaye, S. S. *et al.* Venus' spectral signatures and the potential for life in the clouds. *Astrobiology* **18**, 1181–1198 (2018).
9. Izenberg, N. R. *et al.* The Venus Life Equation. *Astrobiology* <http://doi.org/10.1089/ast.2020.2326> (In press, 2021).
10. Sagan, C. The planet Venus. *Science* **133**, 849–858 (1961).
11. Cockell, C. S. Life on Venus. *Planet. Space Sci.* **47**, 1487–1501 (1999).
12. Bains, W. *et al.* Phosphine on Venus cannot be explained by conventional processes. Preprint at <http://arxiv.org/abs/2009.06499> (2020).

13. Seager, S. *et al.* The Venusian lower atmosphere haze as a depot for desiccated microbial life: A proposed life cycle for persistence of the Venusian aerial biosphere. *Astrobiology* <http://doi.org/10.1089/ast.2020.2244> (In press, 2020).
14. Stevenson, A. *et al.* *Aspergillus penicillioides* differentiation and cell division at 0.585 water activity. *Environ. Microbiol.* **19**, 687–697 (2017).
15. Meisner, A. *et al.* Soil microbial legacies differ following drying-rewetting and freezing-thawing cycles. *ISME J.* **15**, 1207–1221 (2021).
16. Stevenson, A. *et al.* Multiplication of microbes below 0.690 water activity: implications for terrestrial and extraterrestrial life. *Environ. Microbiol.* **17**, 257–277 (2015).
17. Benner, S. A., Ricardo, A. & Carrigan, M. A. Is there a common chemical model for life in the universe? *Curr. Opin. Chem. Biol.* **8**, 672–689 (2004).
18. Schleper, C. *et al.* *Picrophilus* gen. nov., fam. nov.: a novel aerobic, heterotrophic, thermoacidophilic genus and family comprising archaea capable of growth around pH 0. *J. Bacteriol. Res.* **177**, 7050–7059 (1995).
19. Clegg, S. L., Brimblecombe, P. & Wexler, A. S. Thermodynamic model of the system  $\text{H}^+ - \text{NH}_4^+ - \text{SO}_4^{2-} - \text{NO}_3^- - \text{H}_2\text{O}$  at tropospheric temperatures. *J. Phys. Chem. A* **102**, 2137–2154 (1998).
20. Gmitro, J. I. & Vermeulen, T. Vapor-liquid equilibria for aqueous sulfuric acid. *AIChE J.* **10**, 740–746 (1964).
21. Gmitro, J. I. & Vermeulen, T. *Vapor-Liquid Equilibria for Aqueous Sulfuric Acid* (Technical Report No. UCRL-10886; TID-4500) (Ernest Orlando, Lawrence Berkeley National Laboratory, Berkeley, CA, USA, 1963) <https://doi.org/10.2172/876220>.
22. Wilson, R. E. Humidity control by means of sulfuric acid solutions, with critical compilation of vapor pressure data. *J. Indust. Eng. Chem.* **13**, 326–331 (1921).
23. Seiff, A. *et al.* Models of the structure of the atmosphere of Venus from the surface to 100 kilometers altitude. *Adv. Space Res.* **5**, 3–58 (1985).
24. Ignatiev, N. I. *et al.* Water vapour in the lower atmosphere of Venus: A new analysis of optical spectra measured by entry probes. *Planet. Space Sci.* **45**, 427–438 (1997).
25. Bertaux, J. L. *et al.* SPICAV on Venus Express: Three spectrometers to study the global structure and composition of the Venus atmosphere. *Planet. Space Sci.* **55**, 1673–1700 (2007).
26. Gao, P., Zhang, X., Crisp, D., Bardeen, C. G. & Yung, Y. L. Bimodal distribution of sulfuric acid aerosols in the upper haze of Venus. *Icarus* **231**, 83–98 (2014).
27. Carslaw, K. S., Peter, T., & Clegg, S. L. Modeling the composition of liquid stratospheric aerosols. *Rev. Geophys.* **35**, 125–154 (1997).
28. Martin, S. T. Phase transitions of aqueous atmospheric particles. *Chem. Rev.* **100**, 3403–3454 (2000).
29. Zhang, X., Liang, M. C., Mills, F. P., Belyaev, D. A. & Yung, Y. L. Sulfur chemistry in the middle atmosphere of Venus. *Icarus* **217**, 714–739 (2012).

30. Arney, G. *et al.* Spatially resolved measurements of H<sub>2</sub>O, HCl, CO, OCS, SO<sub>2</sub>, cloud opacity, and acid concentration in the Venus near-infrared spectral windows. *J. Geophys. Res. Planets* **119**, 1860–1891 (2014).
31. Krasnopolsky, V. A. Vertical profiles of H<sub>2</sub>O, H<sub>2</sub>SO<sub>4</sub>, and sulfuric acid concentration at 45–75 km on Venus. *Icarus* **252**, 327–333 (2015).
32. Hallsworth, J. E. Wooden owl that redefines Earth's biosphere may yet catapult a fungus into space. *Environ. Microbiol.* **21**, 2202–2211 (2019).
33. Koop, T., Luo, B., Biermann, U. M., Crutzen, P. J. & Peter, T. Freezing of HNO<sub>3</sub>/H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O solutions at stratospheric temperatures: Nucleation statistics and experiments. *J. Phys. Chem. A* **101**, 1117–1133 (1997).
34. Ball, P. Water is an active matrix of life for cell and molecular biology. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 13327–13335 (2017).
35. Brown, A. D. *Microbial Water Stress Physiology* (John Wiley & Sons, Chichester, United Kingdom, 1990).
36. Chin, J. P. Solutes determine the temperature windows for microbial survival and growth. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 7835–7840 (2010).
37. Hallsworth, J. E., Heim, S. & Timmis, K. N. Chaotropic solutes cause water stress in *Pseudomonas putida*. *Environ. Microbiol.* **5**, 1270–1280 (2003).
38. de Lima Alves, F. *et al.* Concomitant osmotic and chaotropicity-induced stresses in *Aspergillus wentii*: compatible solutes determine the biotic window. *Curr. Genet.* **61**, 457–477 (2015).
39. Santos, R., de Carvalho, C. C. C. R., Stevenson, A., Grant, I. R. & Hallsworth, J. E. Extraordinary stress-tolerance of mycobacteria. *Environ. Microbiol.* **7**, 746–764 (2015).
40. Oleinikova, A., Smolin, N., Brovchenko, I., Geiger, A. & Winter, R. Formation of spanning water networks on protein surfaces via 2d percolation transition. *J. Phys. Chem. B* **109**, 1988–1998 (2005).
41. Arcangeli, C., Bizzarri, A. R. & Cannistraro, S. Role of interfacial water in the molecular dynamics-stimulated dynamical transition of plastocyanin. *Chem. Phys. Lett.* **291**, 7–14 (1998).
42. Roos-Serote, M. *et al.* Proximate humid and dry regions in Jupiter's atmosphere indicate complex local meteorology. *Nature* **405**, 158–160 (2000).
43. Seiff, A. *et al.* Thermal structure of Jupiter's atmosphere near the edge of a 5- $\mu$ m hot spot in the north equatorial belt. *J. Geophys. Res. Planets* **103**, 22857–22889 (1998).
44. Wong, M. H., Mahaffy, P. R., Atreya, S. K., Niemann, H.B. & Owen, T. C. Updated Galileo probe mass spectrometer measurements of carbon, oxygen, nitrogen, and sulfur on Jupiter. *Icarus* **171**, 153–170 (2004).
45. Li, C. *et al.* The water abundance in Jupiter's equatorial zone. *Nat. Astron.* **4**, 609–616 (2020).

46. Roos-Serote, M., Atreya, S. K., Wong, M. K. & Drossart, P. On the water abundance in the atmosphere of Jupiter. *Planet. Space Sci.* **52**, 397–414 (2004).
47. Schuerger, A. C., Richards, J. T., Newcombe, D. A. & Venkateswaran, K. Rapid inactivation of seven *Bacillus* spp. under simulated Mars UV irradiation. *Icarus* **181**, 52–62 (2006).
48. Withers, P. & Smith, M. D. Atmospheric entry profiles from the Mars exploration rovers Spirit and Opportunity. *Icarus* **185**, 133–142 (2006).
49. Fedorova, A. A. *et al.* Stormy water on Mars: The distribution and saturation of atmospheric water during the dusty season. *Science* **367**, 297–300 (2020).
50. Murphy, D. M. & Koop, T. Review of the vapour pressures of ice and supercooled water for atmospheric applications. *Q. J. R. Meteorol. Soc.* **131**, 1539–1565 (2005).
51. Kreidberg, L. *et al.* A precise water abundance measurement for the hot Jupiter WASP-43b. *Astrophys. J. Lett.* **793**, L27 (2014).
52. Villanueva, G. *et al.* No phosphine in the atmosphere of Venus. Preprint at arXiv:2010.14305 (2020).
53. Snellen, I. A. G., Guzman-Ramirez, L., Hogerheijde, M. R., Hygate, A. P. S. & van der Tak, F. F. S. Re-analysis of the 267 GHz ALMA observations of Venus - No statistically significant detection of phosphine. *Astron. Astrophys.* **644**, L2 (2020).
54. Witze, A. 'Life on Venus' claim faces strongest challenge yet. *Nature* **590**, 19–20 (2021).
55. Greene, T. P. *et al.* Characterizing transiting exoplanet atmospheres with JWST. *Astrophys. J.* **817**, 17 (2016).
56. Hoffmann, E. H. *et al.* An advanced modeling study on the impacts and atmospheric implications of multiphase dimethyl sulfide chemistry. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 11776–11781 (2016).
57. Attard, E. *et al.* Effects of atmospheric conditions on ice nucleation activity of *Pseudomonas*. *Atmos. Chem. Phys.* **12**, 10667–10677 (2012).
58. Warneck P. & Williams J. The atmospheric aerosol. In: *The Atmospheric Chemist's Companion* pp. 127–187 (Springer, Dordrecht, 2012).
59. Bohren, C. F. & Clothiaux, E. *Fundamentals of Atmospheric Radiation*. Vol. 25 (Wiley-Vch, Weinheim, 2006).
60. Fleming, E. L., Chandra, S., Shoeberl, M. R. & Barnett, J. J. Monthly mean global climatology of temperature, wind, geopotential height, and pressure for 0-120 km. *NASA Tech. Memo., TM-100697* 1–96 (NASA, Washington, DC, 1988).
61. Wagner, W. & Pruss, A. International equations for the saturation properties of ordinary water substance. Revised according to the international temperature scale of 1990. *Addendum to J. Phys. Chem. Ref. Data* **16**, 893 (1987). *J. Phys. Chem. Ref. Data* **22**, 783-787.
62. Wexler, A. S. & Clegg, S. L. Atmospheric aerosol models for systems including the ions  $\text{H}^+$ ,  $\text{NH}_4^+$ ,  $\text{Na}^+$ ,  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ , and  $\text{H}_2\text{O}$ . *J. Geophys. Res.* **107**, D14 (2002).

63. Jia, S. Technical note: Comparison and interconversion of pH based on different standard states for aerosol acidity characterization. *Atmos. Chem. Phys.* **18**, 11125–11133 (2018).
64. International Union of Pure and Applied Chemistry. *IUPAC Compendium of Chemical Terminology, 2nd ed. (the "Gold Book")*. Compiled by McNaught, A. D. & Wilkinson, A. Blackwell Scientific Publications, Oxford (1997). Online version (2012-) created by S. J. Chalk. ISBN 0-9678550-9-8. <https://doi.org/10.1351/goldbook>.
65. Lamb, D., & Verlinde, J. *Physics and Chemistry of Clouds* (Cambridge University Press, Cambridge, 2011).
66. Camuffo, D. Physics of drop formation and micropore condensation. In: *Microclimate for Cultural Heritage Conservation, Restoration, and Maintenance of Indoor and Outdoor Monuments* (2ed. Camuffo, D.) 165–201 (Elsevier, 2014).
67. Wix, A., Brachert, L., Sinanis, S. & Schaber, K. A simulation tool for aerosol formation during sulphuric acid absorption in a gas cleaning process. *J. Aerosol Sci.* **41**, 1066–1079 (2010).
68. Gardner, J. A. *et al.* Measurement of the mass accommodation coefficient of SO<sub>2</sub> (g) on water droplets. *J. Geophys. Res.* **92**, 10887–10895 (1987).
69. Schulze-Makuch, D., Grinspoon, D. H., Abbas, O., Irwin, L. N. & Bullock, M. A. A sulfur-based survival strategy for putative phototrophic life in the Venusian atmosphere. *Astrobiology* **4**, 11–18 (2004).
70. Dartnell, L. R. *et al.* Constraints on a potential aerial biosphere on Venus: I. Cosmic rays. *Icarus* **257**, 396–405 (2015).
71. Winston, P. W. & Bates, D. H. Saturated solutions for the control of humidity in biological research. *Ecology* **41**, 232–237 (1960).
72. Lee, C. J. D. *et al.* NaCl-saturated brines are thermodynamically moderate, rather than extreme, microbial habitats. *FEMS Microbiol. Rev.* **42**, 672–693 (2018).
73. Harrison J. P., Dobinson L., Freeman K., McKenzie R., Wyllie D. *et al.* Aerobically respiring prokaryotic strains exhibit a broader temperature–pH–salinity space for cell division than anaerobically respiring and fermentative strains. *J. R. Soc. Interface* **12**, 20150658 (2015).
74. Benison, K. C., O'Neill, W. K., Blain, D. & Hallsworth, J. E. Water activities of acid brine lakes approach the limit for life. *Astrobiology* [doi.org/10.1089/ast.2020.2334](https://doi.org/10.1089/ast.2020.2334) (In press, 2021).
75. Moger-Reischer, R. Z. & Lennon, J. T. Microbial ageing and longevity. *Nat. Rev. Microbiol.* **17**, 679–690 (2019).
76. Khaleque, H. N., Kaksonen, A. H., Boxall, N. J. & Watkin, E. L. J. Chloride ion tolerance and pyrite bioleaching capabilities of pure and mixed halotolerant, acidophilic iron- and sulfur-oxidizing cultures. *Miner. Eng.* **120**, 87–93 (2018).
77. Khaleque, H. N., *et al.* Genome-based classification of two halotolerant extreme acidophiles, *Acidihalobacter prosperus* V6 (=DSM 14174 =JCM 32253) and '*Acidihalobacter ferrooxidans*' V8 (=DSM 14175 =JCM 32254) as two new species, *Acidihalobacter aeolianus* sp. nov. and

490 *Acidihalobacter ferrooxydans* sp. nov., respectively. *Int. J. Syst. Evol. Microbiol.* **69**, 1557–1565  
491 (2019).

492 78. Hallsworth, J. E. *et al.* Limits of life in MgCl<sub>2</sub>-containing environments: Chaotropicity defines  
493 the window. *Environ. Microbiol.* **9**, 801–813 (2007).

494 79. Stevenson, A., Hamill, P. G., Dijksterhuis, J. & Hallsworth, J. E. Water-, pH- and temperature  
495 relations of germination for the extreme xerophiles *Xeromyces bisporus* (FRR 0025), *Aspergillus*  
496 *penicillioides* (JH06THJ) and *Eurotium halophilicum* (FRR 2471). *Microb. Biotechnol.* **10**, 330–  
497 340 (2017).

498 80. Rosso, L., Lobry, J. R., Bajard, S. & Flandrois, J. P. Convenient model to describe the  
499 combined effects of temperature and pH on microbial growth. *Appl. Environ. Microbiol.* **61**, 610–  
500 616 (1995).

501 81. Beale, E. Confidence regions in non-linear estimation. *J. R. Stat. Soc. Series B Stat.*  
502 *Methodol.* **22**, 41–88 (1960).

503 82. Fu, W. & Zhang, X. Global phosphorus dynamics in terms of phosphine. *NPJ Clim. Atmos.*  
504 *Sci.* **3**, 51 (2020).

505 83. Rozenberg, M., Loewenschuss, A. & Nielsen, C. J. Hydrogen bonding in the sulfuric acid-  
506 methanol-water system: A matrix isolation and computational study. *J. Phys. Chem. A* **119**,  
507 2271–2280 (2015).

508 84. Rozenberg, M., Loewenschuss, A. & Nielsen, C. J. H-bonding of sulfuric acid with its  
509 decomposition products: An infrared matrix isolation and computational study of the  
510 H<sub>2</sub>SO<sub>4</sub>·H<sub>2</sub>O·SO<sub>3</sub> complex. *J. Phys. Chem. A* **120**, 3450–3455 (2016).

511 85. Stevenson, A. & Hallsworth, J. E. Water and temperature relations of Actinobacteria.  
512 *Environ. Microbiol. Rep.* **6**, 744–755 (2014).

513 86. Ball, P. & Hallsworth, J. E. Water structure and chaotropicity: their uses, abuses, and  
514 biological implications. *Phys. Chem. Chem. Phys.* **17**, 8297–8305 (2015).

515 87. Cray, J. A. Chaotropicity: A key factor in product tolerance of biofuel-producing  
516 microorganisms. *Curr. Opin. Biotechnol.* **33**, 228–259 (2015).

517 88. Roy, C. *et al.* Microbiome and ecology of hot spring-microbialite system on the Trans-  
518 Himalayan plateau. *Sci. Rep.* **10**, 5917 (2020).

519 89. Stevenson, A. *et al.* Glycerol enhances fungal germination at the water-activity limit for life.  
520 *Environ. Microbiol.* **19**, 947–967 (2017).

521 90. Williams, J. P. & Hallsworth, J. E. Limits of life in hostile environments: no barriers to  
522 biosphere function? *Environ. Microbiol.* **11**, 3292–3308 (2009).

523 91. Wakisaka, A. & Matsuura, K. Microheterogeneity of ethanol-water binary mixtures observed  
524 at the cluster level. *J. Mol. Liq.* **129**, 25–32 (2006).

525 92. Rozenberg, M., Loewenschuss, A. & Nielsen, C. J. Hydrogen bonding in the sulfuric acid–  
526 methanol–water system: a matrix isolation and computational study. *J. Phys. Chem. A* **119**,  
527 2271–2280 (2015).

93. Zhao, H., Zhanga, Q. & Du, L. Hydrogen bonding in cyclic complexes of carboxylic acid–sulfuric acid and their atmospheric implications. *RSC Adv.* **6**, 71733–71743 (2016).
94. National Oceanic and Atmospheric Administration. *U.S. Standard Atmosphere* (Governmental Printing Office, Washington, DC, 1976).

**Correspondence and requests for materials** should be addressed to J.E.H.

## Acknowledgements

We are grateful to Simon L. Clegg (University of East Anglia, England, UK) for helpful discussions on the use of the E-AIM model at low water-activity and the provision of some code; Charles S. Cockell (University of Edinburgh, Scotland, UK), Dmitry Y. Sorokin (Winogradsky Institute of Microbiology, Russia), and Antonio Ventosa (University of Seville, Spain) for providing information about thermotolerance of halophiles; Mark S. Marley (NASA Ames Research Center, CA, USA) for information on Jupiter and exoplanets; Abel Méndez (University of Puerto Rico, Puerto Rico) for inputs relating to analysis of Earth’s atmosphere; to Jean R. Lobry (University of Lyons, France) who helped with use of the cardinal pH model; Nicholas J. Tosca (University of Cambridge, England, UK) for discussions about thermodynamic properties of aqueous sulfuric acid solutions; and Elizabeth L. J. Watkin (Curtin University, Australia) who provided information about stress tolerance of *Acidihalobacter*. JEH was funded by the Biotechnology and Biological Sciences Research Council (BBSRC, United Kingdom) project BBF003471; MPZ was supported by projects PID2019-104205GB-C21 of Ministry of Science and Innovation and MDM-2017-0737 Unidad de Excelencia “María de Maeztu”- Centro de Astrobiología (CSIC-INTA) (Spain); and OVG was supported by the Centre of Environmental Biotechnology Project (grant 810280) funded by the European Regional Development Fund (ERDF) through the Welsh Government.

## Author contributions

J.E.H., P.B., and M.P.Z. conceived the study; J.E.H., C.P.M., T.K., and M.P.Z. designed the approach; all authors obtained and analysed the data (T.K., M.P.Z., J.E.H., and J.B. for water activity of H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O mixtures; C.P.M. for the Martian and Jovian atmospheres and relative humidity of Venusian atmosphere; T.K., C.P.M., and J.E.H. for the Earth case study; T.K., C.P.M., J.E.H., M.P.Z., and J.B. for quantification of sulphuric acid concentration and water activity of the droplets in Venusian clouds; J.E.H., T.D.D., and O.V.G. for acidity- and water-activity limits-of-life on Earth; M.D., P.B., and J.E.H. for activities of sulphuric acid on the cellular system; and J.E.H., T.D.D., C.P.M., M.D., M.P.Z., J.M.T., T.K., J.B., and P.B. for determination of habitability for Venus’ acid clouds); T.K., C.P.M., J.E.H., T.D.D., M.D., and M.P.Z. constructed the displays; J.E.H. produced an initial draft of the manuscript; all authors contributed to writing the final manuscript.



## Competing interests

The authors declare no competing interests.

## Figure legends

**Figure 1.** Map of water activity of liquid  $\text{H}_2\text{SO}_4$ - $\text{H}_2\text{O}$  mixtures as a function of temperature and sulphuric acid concentration (in weight percent; i.e., %, w/w) over the temperature range pertinent to active life (-40 and 130°C). Values were calculated from Gmitro and Vermeulen<sup>20,21</sup> vapour pressure data (see Supplementary Table 1 and Methods of the current manuscript). The colour scale indicates water-activity values from 0 to 1. Several water activity iso-lines are shown: at a water activity of 0.585 (limit-for-active terrestrial life, which was observed at 24°C and pH 6.1<sup>ref. 14</sup>; black triangle) and extrapolated to other conditions (black iso-line), and several iso-lines at very low water-activity values of 0.01, 0.001, and 0.0001 (thin rose-colour lines, right-hand side). The white square (left-hand side) indicates the tolerance limit for the most-extreme acidophile at incubation temperature of 60°C and pH -0.06<sup>ref. 18</sup> (which is equivalent to 11.5% (w/w) sulphuric acid at 60°C); this pH limit extrapolated to other conditions (white iso-line). As no microbes are known to grow at 0.585 or pH -0.06 at temperature other than the original culture conditions (black triangle and white square, respectively), the assumption that life might be plausible under other conditions has not been substantiated so each iso-line acts as a conservative indicator of notional biophysical limits for life beyond that black triangle or white square. The grey circles show the conditions of Venus' clouds determined in this study; for details see main text and Table 1.

**Figure 2.** Water activity and relative humidity of the Venus atmosphere in the region where temperatures are in the range of possible biological interest (-40°C to +130°C). The uncertainty in altitude is smaller than the size of the data-point markers. The uncertainty in water activity is  $\pm 30\%$  of the value, as described in the text.

**Figure 3.** Schematic diagram to show the implications of the water-activity values of  $\text{H}_2\text{SO}_4$ - $\text{H}_2\text{O}$  mixtures, including those found within the cloud layer of Venus, for cellular terrestrial-type life. The narrow red zone (which resembles thick a red line) indicates the water activity of the  $\text{H}_2\text{SO}_4$ - $\text{H}_2\text{O}$  droplets of the lower Venus cloud layer (altitude about 40 to 70 km) within the temperature range that is consistent with habitability, based on knowledge of Earth's microbial biosphere (Table 1; refs.<sup>4,14,18</sup>). **a.** The approximate sulphuric acid concentrations at 25°C relating to the water activities given on the right were calculated using E-AIM<sup>19</sup> and Gmitro and Vermeulen<sup>20,21</sup> at 25°C, and the average concentration is given here (see Methods). **b.** For details, see main text. **c.** The thermoacidophile, *P. torridus*, is not known to grow at lower water activity than that of

11.5% (w/w) sulphuric acid (about 0.950<sup>19</sup> - pink arrow; see Methods). For further information about H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O mixtures, see Supplementary 'Text for Figure 3'. **d.** This polyextremophile is halotolerant and acidophilic but grows only down to pH 2 at high NaCl at 30°C, with a culture-medium water-activity of 0.955 (grey arrow; see also main text). **e.** These are two haloarchaea able to grow down to 0.635 water activity (green arrow) at high salt concentrations<sup>3</sup> and the acidotolerant xerophile/halophile *A. penicilliioides* can grow down to 0.585 water activity (black arrow) at high glycerol concentration<sup>14</sup>. These microbes cannot tolerate high sulphuric acid concentrations but are shown here as they represent the water-activity limits for active prokaryote and eukaryote life, respectively (N.B. at 24°C, 0.585 water activity is equivalent to 39.0% (w/w) sulphuric acid [pH -1.55] according to Clegg *et al.*<sup>19</sup>).

**Figure 4.** Water activity in the Jupiter atmosphere over the range of temperatures suitable for life. These temperatures correspond to pressures of 2.5 to 10 bars. Temperature and pressure are taken from the Galileo probe data<sup>23</sup>. A constant water-vapour mixing ratio is assumed to be set by the solar ratio of O/H<sup>46</sup>. The water activity decreases for temperatures less than 0°C due to the reduction in water activity of ice with temperature.

**Figure 5.** Average water-vapour mixing ratio profiles in Earth's atmosphere under cloud-free conditions. At lower altitude, three different profiles represent polar, mid-latitude, and tropical troposphere<sup>58</sup> (solid blue, green, and red circles, respectively), which were then interpolated (open circles) to the average profile in the stratosphere and mesosphere<sup>59</sup> (solid black circles); see Methods.

**Figure 6.** Relative-humidity and water-activity ranges at different altitudes in Earth's atmosphere for cloud-free conditions and in clouds: **(a)** maximum and minimum relative-humidity averages for January and July reference atmosphere for cloud-free conditions in polar, mid-latitude, and tropical regions (blue, green, and red, respectively), calculated using temperature-pressure data from the COSPAR International Reference Atmosphere<sup>60</sup> and the vapour-mixing ratio profiles from Supplementary Figure 5, Methods; **(b)** typical in-cloud water-activity values. Liquid-water clouds, for example fog and cumulus clouds (Cu), are indicated by the magenta bar (lower right). Ice clouds, for example cirrus clouds (Ci), type-II polar stratospheric clouds (PSC), and polar mesospheric clouds (PMC), are indicated as orange boxes. For further information on cloud types, conditions for formation, and location, see Supplementary Table 3. Between about 26 and 85 km, it is too dry for clouds to form and persist.

**Figure 7.** Generalized exoplanet water-activity analysis. Temperature and pressure profile that corresponds to a water activity of 0.585 - the limit for active life<sup>14</sup> - for a water-vapour mixing ra-

640 tio set by the solar ratio of O/H. For values to the right of the curve, the values of water activity  
641 are less than the 0.585 water-activity limit while for values to the left of the curve the water activi-  
642 ty is greater than this limit. The lowest temperature considered is  $-59^{\circ}\text{C}$ ; below that value the  
643 water activity of ice is less than 0.585.

644

645

646

647

| Temperature<br>(°C) <sup>b</sup> | Altitude above<br>mean ground<br>level (km) <sup>b</sup> | Pressure<br>(bars) <sup>b</sup> | Relative<br>humidity<br>(%) <sup>c</sup> | Water activity <sup>d</sup><br>of cloud<br>droplets | Sulphuric acid<br>concentration of<br>cloud droplets<br>(%, w/w) <sup>e</sup> |
|----------------------------------|--|---------------------------------|--|---|---|
| -40                              | 68.80  | 0.047                           | 0.1397                                   | 0.001397  | 79.6  |
| -35                              | 67.02  | 0.067                           | 0.1803                                   | 0.001803  | 79.3  |
| -30                              | 65.02  | 0.097                           | 0.3051                                   | 0.003051  | 78.1  |
| -25                              | 63.40  | 0.131                           | 0.3692                                   | 0.003692  | 77.8  |
| -20                              | 62.30  | 0.158                           | 0.3235                                   | 0.003235  | 78.6  |
| -15                              | 61.12  | 0.197                           | 0.2847                                   | 0.002847  | 79.3  |
| -10                              | 59.94  | 0.238                           | 0.2440                                   | 0.002440  | 80.2  |
| -5                               | 59.09  | 0.276                           | 0.1982                                   | 0.001982  | 81.1  |
| 0                                | 58.32  | 0.313                           | 0.1605                                   | 0.001605  | 82.1  |
| 5                                | 57.60  | 0.354                           | 0.1296                                   | 0.001296  | 83.1  |
| 10                               | 56.93  | 0.394                           | 0.1043                                   | 0.001043  | 84.1  |
| 15                               | 56.39  | 0.430                           | 0.0831                                   | 0.000831  | 85.2  |
| 20                               | 55.87  | 0.466                           | 0.0664                                   | 0.000664  | 86.4  |
| 25                               | 55.40  | 0.502                           | 0.0533                                   | 0.000533  | 87.5  |
| 30                               | 54.92  | 0.538                           | 0.0430                                   | 0.000430  | 88.7  |
| 35                               | 54.44  | 0.579                           | 0.0351                                   | 0.000351  | 89.8  |
| 40                               | 53.97  | 0.619                           | 0.0288                                   | 0.000288  | 90.9  |
| 45                               | 53.48  | 0.666                           | 0.0239                                   | 0.000239  | 91.9  |
| 50                               | 52.99  | 0.712                           | 0.0199                                   | 0.000199  | 92.9  |
| 55                               | 52.50  | 0.764                           | 0.0167                                   | 0.000167  | 93.9  |
| 60                               | 52.02  | 0.815                           | 0.0140                                   | 0.000140  | 94.7  |
| 70                               | 50.87  | 0.952                           | 0.0104                                   | 0.000104  | 96.1  |
| 80                               | 49.67  | 1.061                           | 0.0075                                   | 0.000075  | 97.2  |
| 90                               | 48.42  | 1.308                           | 0.0061                                   | 0.000061  | 97.9  |
| 100                              | 46.99  | 1.558                           | 0.0048                                   | 0.000048  | 98.4  |
| 110                              | 45.40  | 1.891                           | 0.0040                                   | 0.000040  | 98.8  |
| 120                              | 43.67  | 2.316                           | 0.0034                                   | 0.000034  | 99.1  |
| 130                              | 42.06  | 2.285                           | 0.0030                                   | 0.000030  | 99.2  |

649 a. Based on the assumptions that droplets are in equilibrium with the atmospheric relative humidity and  
650 that the primary sulphate species is sulphuric acid.

651 b. Altitude, temperature, and pressure values are from entry probe data with uncertainties of  $\pm 1$  km,  $\pm 1$  K,  
652  $\pm 5\%$ , respectively<sup>23</sup>.

653 c. Values were computed using the mixing ratio of water from observations as parameterised by Gao *et*  
654 *al.*<sup>26</sup>. Uncertainty, based on reported uncertainties in atmospheric profile, is  $\pm 30\%$  of the value.

655 d. Derived by dividing relative humidity by 100, based on the assumption that droplets are in equilibrium  
656 with the atmospheric relative humidity. Each value is pertinent to the stated temperature (column 1).  
657 Uncertainty, based on reported uncertainties in atmospheric profile, is  $\pm 30\%$  of the value.

658 e. Values, stated to one decimal place, were derived from interpolations of the data of Gmitro and  
659 Vermeulen<sup>20 21</sup> to the water-activity values in column 5, based on the assumption that the primary  
660 sulphate species is sulphuric acid (see Methods).

661

## Methods

**Water activity of  $\text{H}_2\text{SO}_4$ - $\text{H}_2\text{O}$  mixtures.** All solutes/co-solvents depress the water activity of solute- or co-solvent-water mixtures. We sought to identify datasets and models that enable quantification of the water-activity values of  $\text{H}_2\text{SO}_4$ - $\text{H}_2\text{O}$  mixtures over a range of sulphuric acid concentrations and a range of temperatures. For this purpose, we used two independent, semi-empirical thermodynamic models. Gmitro and Vermeulen<sup>20,21</sup> used experimental data to provide a comprehensive model dataset for vapour pressures of water, sulphuric acid and sulphur trioxide of aqueous sulphuric acid solutions from 10 to 100% (w/w) at temperatures from -50 to 400°C. These were tabulated in a supplement to the paper<sup>20,21</sup>, and we extracted the water-vapour pressures from -40 to 130°C. These data were then converted to water activity by dividing them by the water-vapour pressure over pure water at the same temperature (the values for pure water were obtained from Murphy and Koop<sup>50</sup> [up to and including 0°C] and Wagner and Pruss<sup>61</sup> [higher than 0°C]).

These water-activity values were used in the current study to produce Figure 1 by interpolation using the contour plot function of OriginPro 2021 (version 9.8.0.200). We also present four iso-lines of constant water activity in Figure 1. For the 0.585 water-activity iso-line, the corresponding sulphuric acid concentrations were obtained by linear interpolation between the nearest water-activity : sulphuric acid-concentration data pairs from Supplementary Table 1. For the 0.01, 0.001, and 0.0001 water-activity iso-lines, linear interpolation between the nearest  $\log(\text{water-activity})$  : sulphuric acid-concentration data pairs were used because of the several order-of-magnitude changes of water activity in that concentration range. The Gmitro and Vermeulen<sup>20,21</sup> data were also used to calculate interpolated sulphuric acid concentrations in Venus cloud droplets from water activities (see below and Table 1), again by linear interpolation between the nearest  $\log(\text{water-activity})$  : sulphuric acid-concentration data pairs.

The E-AIM<sup>19</sup> was employed to calculate water activities at predefined sulphuric acid concentrations and *vice versa*, as well as corresponding pH values. For that purpose, we employed model I (either option 1. 'simple' or option 3. 'aqueous solution') of the online version of E-AIM<sup>62</sup>. E-AIM is valid from -93.2 to +56.9°C (180 to 330 K) and up to sulphuric acid concentrations of about 80% (w/w). For the pH calculations, E-AIM provides the mole fraction-based  $\text{H}^+$  activity coefficient, which was converted to the molality-based activity coefficient<sup>63</sup> and then the molality-based  $\text{H}^+$  activity in solution, from which the molality-based pH (the negative decadal logarithm of  $\text{H}^+$  activity according to IUPAC convention<sup>64</sup>) is derived. E-AIM<sup>19</sup> was used to calculate the pH -0.06 iso-line shown in Figure 1 for temperatures between -40 and 56°C. Between 25 and 56°C, the corresponding sulphuric acid concentrations showed a perfectly linear behaviour, which enabled a linear extrapolation to 130°C. E-AIM<sup>19</sup> was also used to calculate sulphuric acid concentrations in Venus cloud droplets from water activities, for comparison with those calculated from the Gmitro and Vermeulen<sup>20,21</sup> data. Although the agreement is very good for temperatures up to about 25°C, we note that all these values are outside the stated validity range of E-AIM<sup>19</sup> (sul-

phuric acid concentrations less than ~80% [w/w] and water activity less than 0.01). See also Supplementary 'Text relating to validation of water activity for H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O mixtures'.

**Relative humidity and water activity of Venus' atmosphere.** Small droplets within clouds rapidly equilibrate with relative humidity (as shown in this section, below), so it was imperative to quantify relative humidities of the Venusian atmosphere for the altitude/ temperature range that is potentially habitable according to our knowledge of life on Earth. Between about 40 and 70 km altitude, the atmosphere of Venus has temperatures and pressures similar to those of the lower atmosphere of the Earth. To compute the physical properties in this region for Venus, we used the direct measurements of pressure and temperature from entry probes for equatorial latitudes,  $\pm 30^\circ$  <sup>ref. 43</sup>. These datasets report values for every kilometre in altitude and reported uncertainties in this profile are  $\pm 0.15$  km for altitude,  $\pm 5$  K for temperature, and  $\pm 5\%$  for pressure<sup>46</sup>. Day to night temperature differences are only about  $\pm 5$  K <sup>ref. 43</sup>. The water-vapour mixing ratio is taken from the parameterisation of Gao *et al.*<sup>24</sup> which is based on observations from Bertaux *et al.*<sup>25</sup> and the Venera 11, 13, and 14 missions<sup>24</sup>.

Atmospheric relative humidity is a ratio, calculated according to the atmospheric water-vapour partial pressure divided by the saturation vapour pressure of liquid water at the corresponding temperature, which we computed with the parameterisation of Murphy and Koop<sup>50</sup>. The results (Table 1) are shown pictorially in Figure 2 where the uncertainty in altitude is smaller than the size of the data-point markers. The uncertainty in relative humidity was determined by combining the uncertainties in temperature, pressure, and water-vapour mixing ratio. It was dominated by the uncertainty in the water-vapour parameter which, based on the comparison of the fit to observations shown in Gao *et al.*<sup>26</sup>, we estimate to be  $\pm 30\%$  of the value. It is relevant to note that the data directly determine the water-vapour mixing ratio to be 1 ppm at 70–90 km and 30 ppm below 60 km. In the Gao *et al.* parameterisation<sup>26</sup>, the transition is smooth, and the water-vapour mixing ratio decreases sharply above 60 km resulting in a peak in the relative humidity at about 65 km. If instead the water-vapour mixing ratio is held at 30 ppm through the cloud layer until 70 km, the atmospheric relative humidity would rise monotonically to 0.07% at 70 km.

The water activity of the droplets (Table 1) is taken as equivalent to the relative humidity of the atmosphere because the time constant for growth of the droplets due to collisions with the ambient water vapour is short compared to transport times of the droplets. This assumption is common in Venus atmospheric models<sup>29</sup>. The droplets are large enough (radius approximately 1  $\mu\text{m}$ ) that the reduction of water activity due to curvature in the droplet (the Kelvin effect) is negligible<sup>65,66</sup>. The timescale for water-vapour molecules to accrete on a cloud droplet can be estimated from the collision rate as derived from kinetic theory.

In the kinetic approximation, the number of atoms sticking on a droplet of radius  $r$  per unit time is approximately  $\alpha n 4 \pi r^2 (kT/2 \pi m)^{1/2}$  <sup>ref. 67</sup>. Where  $\alpha$  is the mass accommodation

coefficient,  $n$  is the density of gas-phase water molecules in the atmosphere,  $k$  is Boltzmann's constant,  $T$  is the temperature, and  $m$  is the mass of the water molecules. The formula above ignores the correction due to the small mean free path. Over the range of altitudes considered here, the mean free path varies from 0.02 to 1.2  $\mu\text{m}$  which is smaller than, or comparable to the droplet size of about 1- $\mu\text{m}$  radius.

Expressed in terms of the e-folding time, for the droplet to grow in size by a factor of  $e$ , we have  $\tau^{-1} = r^{-1} dr/dt = r^{-1} \alpha (n/p) (mkT/2\pi)^{1/2}$ , where  $p$  is the density of the droplet and ' $dr/dt$ ' is the rate of change of the radius with time,  $t$ . If we evaluate this for representative conditions in the atmospheric profile, pressure  $\sim 1$  atm, temperature  $\sim 0^\circ\text{C}$ , and water-vapour concentration  $\sim 20$  ppm. We obtain a value of  $T = 0.4$  s, for  $\alpha = 1$ . The mass accommodation,  $\alpha$ , is typically assumed to be unity in models of Venus cloud physics<sup>26</sup>. However, Gardner *et al.*<sup>68</sup> reported a lower limit to  $\alpha$  of  $(5.4 \pm 0.6) \times 10^{-2}$  based on laboratory experiments, which gives a value of  $T = 0.8$  s. These values of  $\tau$  can be compared to the time for a 1- $\mu\text{m}$  radius droplet to settle 1 km which is the step size in our atmospheric model. The settling velocity of a 1- $\mu\text{m}$  radius droplet at standard pressure is  $\sim 10^{-3} \text{ cm s}^{-1}$  giving a time to cover 1 km of  $10^8$  seconds. Eddy mixing will be more important than settling in transporting the droplets. Zhang *et al.*<sup>29</sup> give an eddy coefficient,  $D$ , at 60 km of  $4 \times 10^4 \text{ cm}^2 \text{ s}^{-1}$ . Transport across a distance  $Z$  will occur over a timescale of  $\sim Z^2/D$ . For  $Z = 1$  km, this gives a transport time of  $2.5 \times 10^5$  s. This calculation supports the conclusion that the cloud droplets are in balance with the local atmospheric water abundance, and we equate the water activity in the droplets with the atmospheric relative humidity in Table 1. The low values of water activity that we find (Table 1) are consistent with atmospheric models (Figure A4 in <sup>ref. 29</sup>).

**H<sub>2</sub>SO<sub>4</sub> concentration of the droplets in Venus' clouds.** The functional capability of cellular systems is determined by the biophysical and physicochemical conditions to which the cell - not least, the plasma membrane - are exposed assuming that biocidal factors are absent, and nutrients and energy sources are available. Therefore, it is imperative to consider habitability at a scale that is pertinent to the microbial cell and its macromolecular systems rather than confining analyses to a macro-level or planetary scale, or an anthropocentric viewpoint. Factors such as availability of nutrients, energy sources, temperature, cosmic rays, and ultra-violet radiation have been considered elsewhere (e.g., refs. <sup>5,11,13,69,70</sup>), so here we focused on quantifying the sulphuric acid concentration and water activity of the droplets in Venusian clouds.

The sulphuric acid concentration within cloud droplets was calculated assuming that the primary sulphate species is sulphuric acid. We used the Gmitro and Vermeulen<sup>20,21</sup> data to calculate the sulphuric acid concentrations that are in equilibrium with the relative humidity in Venus' atmosphere, indicated by grey circles in Figure 1; see also Table 1. We also used the E-AIM<sup>19</sup>, although the relative-humidity values are below 0.01 water activity (1% relative humidity) which is the lower water-activity limit at which the model runs. The values that we obtained

agreed with those calculated using the Gmitro and Vermeulen<sup>20,21</sup> data, to within  $\pm 1\%$  (w/w) sulphuric acid for temperatures between  $-40^{\circ}\text{C}$  to  $25^{\circ}\text{C}$ . The formation of solids will not occur, so was suppressed in the model. If we assumed that there is an uncertainty of  $\pm 30\%$  in relative humidity, this leads to only minor changes in sulphuric acid concentrations, which were always less than the symbol size of the grey circles in Figure 1.

**Acidity- and water-activity limits-for-active life on Earth.** No individual terrestrial microbe is likely to be capable of metabolism under the combined conditions of 0.585 water activity, extreme acidity, high ultra-violet radiation, and limited nutrients and energy sources. Some archaea can grow down to pH 1 at about  $70$  or  $80^{\circ}\text{C}$ , which is equivalent to a sulphuric acid concentration of about  $1.2\%$  (w/w) according to Clegg *et al.*<sup>19</sup>. Above  $50^{\circ}\text{C}$ , there are no known microbes able to grow (or retain metabolic functions) at or below a water activity of about 0.700 (the water activity of saturated NaCl at  $50^{\circ}\text{C}$  is  $0.745^{71}$ ); see Figure 1A of Lee *et al.*<sup>72</sup> and Figure 1 of Harrison *et al.*<sup>73</sup>. Nevertheless, 0.585 does represent the ultimate water-activity limit based on current knowledge (see above); we therefore show the position of this water-activity value in Figure 1. For further details, see Supplementary ‘Text on biophysical limits for terrestrial microbes’.

The fungus (*A. penicillioides*) that is able to differentiate and grow at 0.585, the lowest water activity for active life<sup>14,32</sup>, is also acidotolerant (see below). At this water activity, differentiation and cell division were observed on a nutrient medium supplemented with  $7.7\text{ M}$  glycerol, at pH 6.1<sup>ref.14</sup>. Low water-activity, low pH habitats in nature are not common. Whereas a recent study of acid-brine lakes (Western Australia) revealed water-activity values as low as 0.714 (pH 1.4), there are no definitive data to indicate microbial activity under these conditions<sup>74</sup>. The lowest water-activity at which proliferation of halophilic prokaryotes has been observed is 0.635, in a NaCl-dominated but  $\text{MgCl}_2$ -rich (bittern) brine<sup>3</sup>. We doubt that any acidophilic species would be capable of metabolism at such hostile water-activity values given the level of energy generation that would be needed to cope with these concomitant extremes of water activity and acidity. Also, the cellular damage caused by this combination of extremes would likely outweigh the capacity for self-repair, resulting in senescence and death<sup>75</sup>. It is possibly for the same reason that the most-extreme xerophiles and halophiles have not evolved to be acidophilic, even though in nature they can be exposed to low-pH conditions (e.g., in acid-brine lakes and bittern brines).

The acid-tolerance limit of the most-acidophilic microbe known (*P. torridus*), detailed above, has been converted to a water-activity value as shown in Figure 3. There is one acidophile that is known to be halotolerant; the bacterium *A. aeolianus*. This microbe is chemolithoautotrophic and capable of growth at  $1283\text{ mM}$  NaCl and pH 2 (adjusted by addition of concentrated sulphuric acid to the nutrient medium;  $30^{\circ}\text{C}$ )<sup>ref. 76,77</sup>. In these studies, the final concentration of sulphuric acid was not empirically determined. Therefore, we used E-AIM<sup>19</sup> to calculate the concentration of sulphuric acid which corresponds to a pH of 2 at  $30^{\circ}\text{C}$ ; this was



0.071% (w/w). Also using E-AIM, we calculated that this concentration of sulphuric acid causes a water-activity reduction of 0.0003 units. We then calculated the water activity of the nutrient medium, which is a sum of the water activity changes caused by sulphuric acid, NaCl, nutrients, and agar. The water-activity reduction caused by 1283 mM NaCl is 0.043 units (see <sup>ref. 78</sup>), and the reduction caused by nutrients and agar in the culture medium is typically 0.002 units. Therefore, the water activity of the medium (1 minus [0.0003+0.043+0.002]) was 0.955 (as shown in Figure 3). Whereas the related bacterium *Acidihalobacter prosperus* grew down to pH 1.5 in a separate experiment, it is not known to be capable of this level of acid tolerance at elevated NaCl<sup>76</sup>.

The 0.585 water-activity limit for *A. penicilliioides* growing at high glycerol concentration (at 24°C; 297 K, see black triangle in Figure 1) would be equivalent to about 39% (w/w) sulphuric acid according to Gmitro and Vermeulen<sup>20,21</sup> and E-AIM<sup>19</sup>, and a molality-based pH value of -1.55 according to E-AIM and IUPAC convention<sup>64</sup>. However, growth of *A. penicilliioides* has only been recorded down to pH 3 (on a citric acid/Na<sub>2</sub>PO<sub>4</sub>-buffered nutrient medium) according to a study of germination over a range of pH (Supplementary Figure 2; <sup>ref. 79</sup>). Therefore, we carried out an extrapolation of the data shown in Supplementary Figure 2 to determine the theoretical pH minimum for growth, using the Cardinal pH Model (Equation 7 from Rosso *et al.*<sup>80</sup>) in R version 4.0.3 (Supplementary Figure 2). From the datapoints in Supplementary Figure 2, the pH beyond which no germ-tube growth occurs (pH<sub>max</sub>) was set at 10; the pH below which no germ-tube growth occurs (pH<sub>min</sub>) was provisionally set at 2; the pH at which that rate of germ-tube growth was optimal (pH<sub>opt</sub>) was set at 6.5; and the optimum rate of germ-tube extension (μ<sub>opt</sub>) was set at 0.0188 mm d<sup>-1</sup> in order to obtain a curve (Rosso *et al.*<sup>80</sup>). The fit of this curve was refined using the sum of square residuals (SSR) formula and then a non-linear minimisation formula, resulting in a SSR value of 1.07x10<sup>-6</sup>. Using this procedure, the minimum pH was found to be 2.3 (Supplementary Figure 2). To assess the confidence of this value, a 95% confidence level was determined using this formula<sup>81</sup>.

$$\frac{\theta}{S(\theta)} \leq S(\hat{\theta}) \left(1 + \frac{p}{n-p} F_{p,n-p}^{\alpha}\right)$$

where α indicates a region of confidence for the value of the parameters with a risk of the first kind that is given by the set of values of parameters such that the sum of the SSR does not exceed a given threshold; *p* is the number of model parameters; *n* is the number of points available in the dataset; and  $\hat{\theta}$  is the vector of values of parameters such that the criterion is minimal. The 95% confidence interval for the minimum pH for growth was pH 1.96 to 2.57.

The pH value 2.3 is equivalent to 0.031% (w/w) sulphuric acid at 24°C according to Clegg *et al.*<sup>19</sup>. Furthermore, the sulphuric acid concentration of 39% (w/w) is about half that of the least concentrated Venus' cloud droplets (see below) and about four times the tolerance limit of the most-acidophilic microorganism known.

The sulphuric acid concentrations shown on the vertical axis of Figure 3 were first calculated using E-AIM<sup>19</sup> and Gmitro and Vermeulen<sup>20,21</sup> at 25°C, and the average concentration was then plotted. Whereas there is some temperature-dependence of the relationship between sulphuric acid concentration and water activity, this is of minor importance on the scale of Figure 3, see Supplementary Figure 1 and Supplementary Tables 1 and 2 for temperature comparisons. The variation in water activity over the entire temperature range of Venus' cloud layer is from 0.0037 (at -25°C) to 0.00003 (at 130°C). On the linear water-activity scale in Figure 3, this variation occurs within the red zone (that appears as a thick red line) and so is in this way indistinguishable.

**Activities of sulphuric acid on the cellular system.** We sought to identify the primary activities of sulphuric acid on the cellular system because these may also act as determinants for habitability of sulphuric acid clouds. Broadly, they fell into three logical categories: inherent properties of the H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O mixtures, biophysical effects on cellular macromolecules (operating at the level of non-covalent interactions), and chemical modification(s) of cellular macromolecules (these operate primarily at the level of covalent bonds). Searches of the literature (physics, chemistry, biochemistry, microbiology) revealed the primary modes-of-action of sulphuric acid (see Supplementary 'Text for Figure 3'). These were used to construct a schematic diagram of habitability for the sulphuric acid clouds of Venus' putative habitable zone (Figure 3). This display shows: properties of H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O mixtures; biophysical effects of sulphuric acid on cellular macromolecules; chemical modifications of cellular macromolecules induced by sulphuric acid; and the known tolerance limits of terrestrial life in relation to acidity (acidophile and polyextremophile) and low water-activity (xerophile/polyextremophile and halophiles). It also shows the sulphuric acid concentration of the putative habitable zone of the Venus clouds (red zone; Figure 3).

**Determination of habitability for Venus' acid clouds.** The notion that phosphine in the Venusian atmosphere might act as a biosignature rests on the fact that no long-lived geological processes on Earth are known to generate it, whereas it may be produced by terrestrial organisms<sup>82</sup>. The question is, however, whether Venus hosts any regimes that might plausibly support life. In the current study, determinations of habitability of Venus' clouds were based on knowledge of:

- activities of sulphuric acid on the cellular system;
- the current understanding that cellular life requires water<sup>16,34,35</sup>;
- biophysical/physicochemical limits-for-life on Earth and the underlying stress mechanisms that ultimately induce cell-system failure;

- the sulphuric acid concentration and water activity of droplets within Venus' clouds at the altitude/temperature range that is potentially habitable, based on knowledge of the functional biosphere on Earth; and

- identification of any thermodynamic/ physicochemical distance (along with the acidity- and water-activity scales) between the metabolic activity-limits-of-terrestrial life and the putative habitat of Venus' clouds. These are summarised in Table 1 and Figure 3.

One must assume that, at the acid concentrations found in Venus' clouds, sulphuric acid molecules would hydrogen-bond to biological macromolecules (Figure 3), if these are at all comparable to hydrophilic proteins in as much as they are water-soluble; certainly, hydrogen bonding of sulphuric acid to water and methanol is possible<sup>83,84</sup>. In this case, solvation would more-closely resemble that in the presence of concentrated macromolecular cryoprotectants such as trehalose, where the role of the protectant co-solvent is more to immobilise the protein and prevent denaturation than to sustain function.

At the acidity limit for terrestrial life, we believe that, paradoxically, it is water activity that is the limiting factor. The water activity of a 11.5% (w/w) sulphuric acid solution according to the Clegg *et al.*<sup>19</sup> thermodynamic model is about 0.95 (we calculated 11.4% [w/w] at 56°C [329 K]; i.e., near the upper limit of the model, and extrapolation of Clegg *et al.*-derived values to 60°C yields 11.5% [w/w]). The majority of microbes, apart from specialist halophiles and xerophiles, have a minimum water-activity for growth in the range 0.920 to 0.950<sup>refs. 4,16,34,85</sup>, and a likely limit in this range is consistent with the growth phenotype and ecology of *Picrophilus* which is not known in saline habitats. The sulphuric acid tolerance of *Picrophilus*, therefore, is likely determined by its limits of xerotolerance rather than tolerance to acidity *per se*. This is analogous to the growth limit of the *Saccharomyces cerevisiae* strains that are able to tolerate the highest levels of ethanol, a cellular stressor that entropically disorders macromolecular systems; i.e., it acts as a chaotrope<sup>86</sup>. The apparent tolerance limit to this chaotropicity also coincides with the lower water-activity limit for growth of this species (see below). It is noteworthy that the highest sulphuric acid tolerance reported for a microbe was exhibited at a high temperature (60°C), which is in the thermophile range; and that some acidophiles even grow up to 80 or 90°C. Whereas chaotropic substances can reduce the growth minima of microbes<sup>36</sup>, diverse lines of evidence suggest that kosmotropic substances (e.g., sulphate ions) can stabilise cellular macromolecules at high temperature and may thereby enable growth at higher temperatures; conversely, increased temperatures likely enable tolerance to high concentrations of kosmotropes (<sup>ref. 87</sup> and references therein;<sup>88</sup>). For the ultimate terrestrial acidophile, water activity and pH may act concomitantly to curtail metabolism.

Other biophysical conditions that appear to limit the functional biosphere on Earth might also be a consequence of insufficient solvent water. For example, the growth limit of *A. penicillioides* at 7.7 M glycerol may be an artefact in as much as the microbe may be capable at growth below the 0.585 water-activity value. In this case, when glycerol greatly predominates

over water, the cell may cease to function. It is noteworthy that other studies of xerophilic fungi in a similar range of glycerol concentrations found that substituting some of the glycerol with a different solute could reduce the water-activity limit for growth<sup>89,90</sup>. Whereas this could have been because the kosmotropicity of the added solute mitigated against the chaotropicity of glycerol<sup>90</sup>, it is also plausible that the partial substitution of glycerol by a different solute meant that a threshold glycerol concentration, where this polyol prevents water acting as the cellular solvent, was not reached.

Similarly, the most ethanol-tolerant strains of *S. cerevisiae* remain active to almost 20% (w/v) (i.e., 28%, v/v) ethanol; concentrations that reduce water activity to a level consistent with their limit of xerotolerance; about 0.900 water activity<sup>87</sup>. Studies of ethanol-water mixtures show qualitative changes in this range, that have been discussed in the context of microheterogeneity<sup>91</sup>, but can equally be considered an indicator that ethanol has begun to displace water as the cellular solvent. The same may be true for the ions that can limit the growth of even the most-extreme halophiles; this may also be why the water-activity limits of extremely halophile prokaryotes (in brines) and extremely xerophilic eukaryotes (in non-saline habitats) converge towards a common value<sup>3</sup>. The issue in each case (brines, sulphuric acid, glycerol, ethanol) seems to be not so much how much co-solvent can the microbial cell tolerate, but how little water remains.

The situation would be further complicated for sulphuric acid by the likely extreme protonation of any protein-like macromolecules, and perhaps by the disruptive ‘salting-out’ effect exerted on proteins by sulphate- and hydrogen sulphate ions, amongst other adverse effects (Figure 3). Might sulphuric acid itself act as a biophilic solvent, though? There seems no reason to rule out this speculative possibility *per se*. Sulphuric acid is capable of forming hydrogen bonds with water molecules, hydroxyl groups and carboxylic acids<sup>92,93</sup>, and it is a polar molecule with a dielectric constant comparable to that of water (around 100 and 80 respectively). But it cannot be expected to mediate macromolecular interactions in the same way that water does. The role of water’s motions and fluctuations on biological macromolecules seems to depend for example on the highly cooperative dynamics created by its three-dimensional hydrogen-bonding network. So, there would then be little justification for extrapolating from the biochemistry of terrestrial organisms at all.

**Analyses of water-activity and habitability for clouds of Jupiter, Mars, and exoplanets.** For Jupiter, the method employed consisted of three steps. First, a measured profile of temperature and pressure (from the Galileo Atmospheric Entry Probe) was used; second, we assumed that the water abundance is set at the value that corresponds to the solar ratio of oxygen to hydrogen; and third, water activity was calculated by computing the atmospheric partial pressure of water from the pressure and water-vapour mixing ratio, and the equilibrium vapour pressure of liquid water from the temperature. Water activity is the ratio of these. Below freezing, when the

calculation indicates that ice is present, the water activity is equal to the ratio of the vapour pressure of ice to the vapour pressure of pure liquid water. This ratio is also how the water activity at Martian temperatures was computed. The method employed for Jupiter can be applied to exoplanets when the temperature and pressure profiles and water abundance are obtained from observations transits of the planet in front of the star.

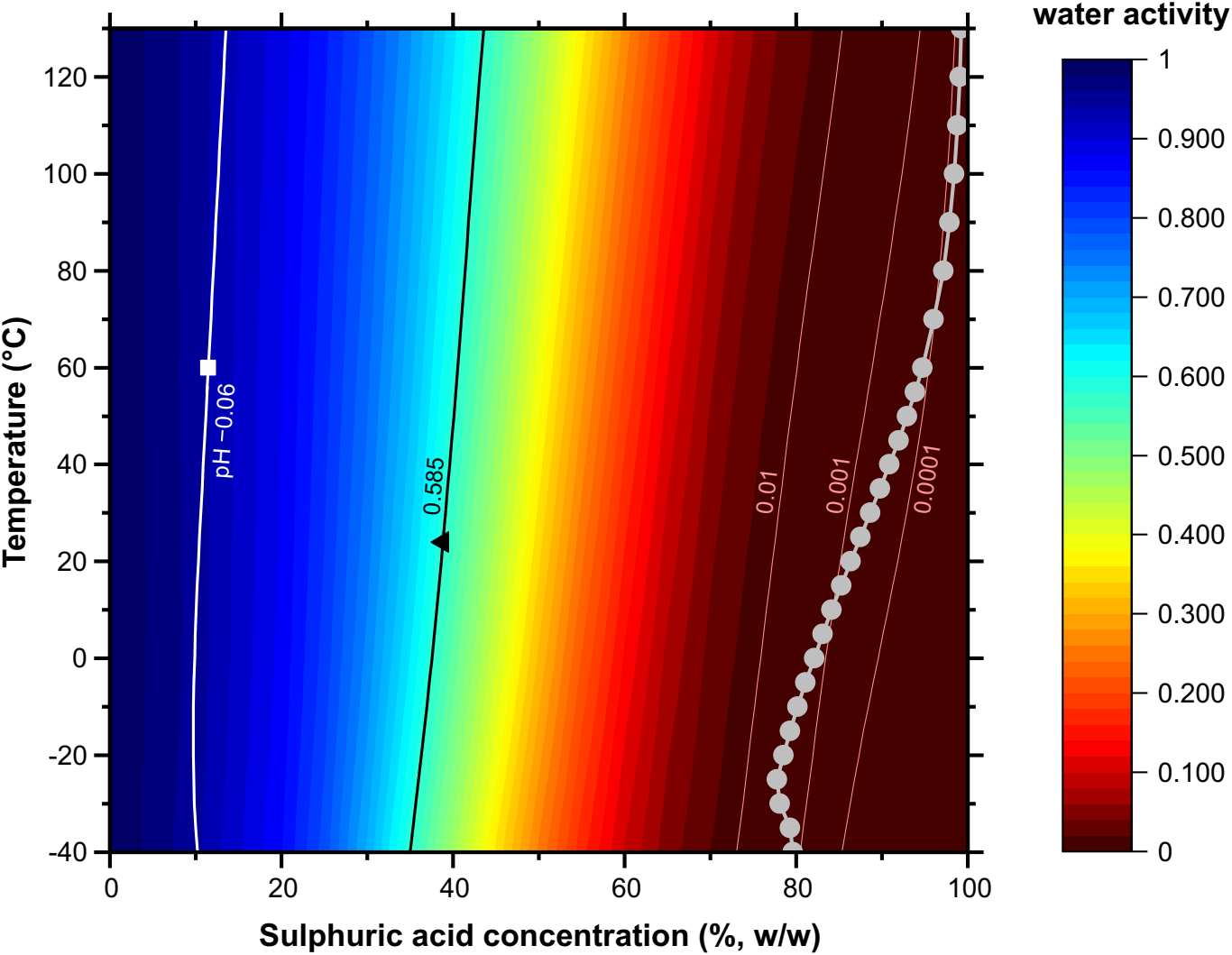
### **Analyses of water activity and habitability of Earth's clouds: A comparative case study.**

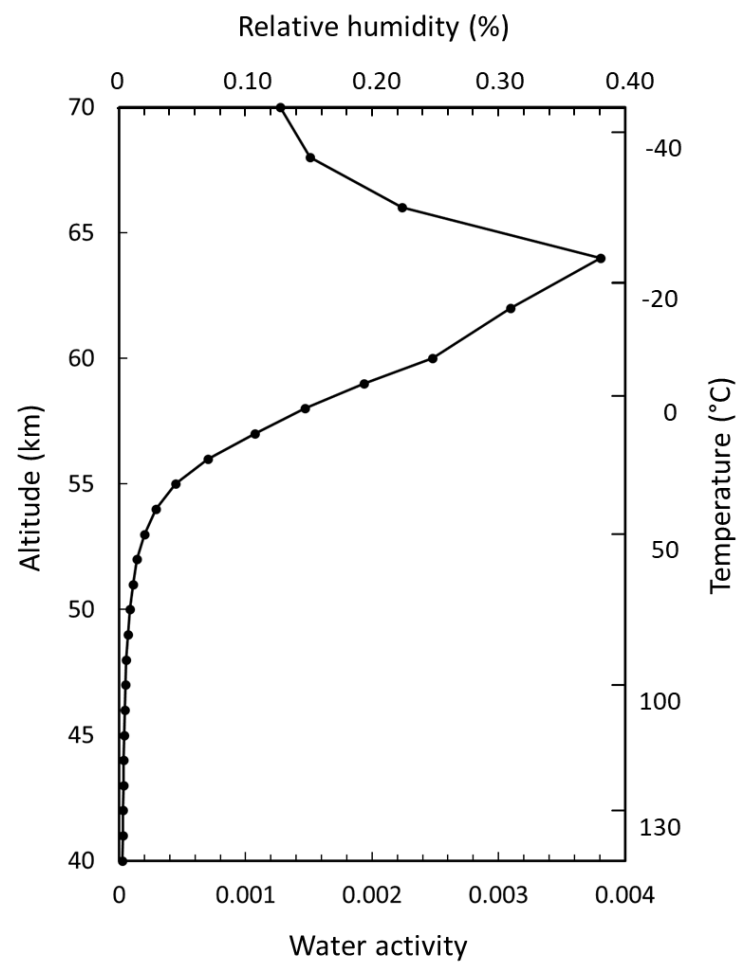
We applied our approach of computing the water activity from temperature and water-vapour abundance data also to Earth's atmosphere and clouds. For this purpose, we used the COSPAR International Reference Atmosphere<sup>60</sup> that acts as a model of the terrestrial atmosphere and is based on empirical temperature-, pressure- and altitude data. Both temperature and water-vapour mixing ratio are highly variably in Earth's atmosphere. This variability is to a large part due to the existence of both land and expansive oceans, differences in solar radiative influx with latitude, and the pronounced diurnal light-dark cycle and annual seasonal cycles. Indeed, thunderstorms and dry heatwaves can occur at the same location within a matter of days. Therefore, we use three representative water-vapour mixing profiles: for the polar, mid-latitude and tropical troposphere (solid blue, solid green, and solid red circles, respectively, in Figure 5)<sup>58</sup> and for altitudes of  $\geq 20$  km, one average profile (solid black circles in Figure 5) which is based on data from Bohren and Clothiaux<sup>59</sup>. As the height of the interface between the troposphere and stratosphere (tropopause) varies with latitude, being lower at the poles and higher in the tropics, we interpolated the tropospheric profiles to the water-vapour mixing ratio at 20 km (open circles in Figure 5).

These water-vapour mixing ratios can then be used together with the COSPAR International Reference Atmosphere<sup>60</sup> average temperature- and pressure profiles to calculate relative humidity and water activity. For this purpose, we extracted temperature-versus-altitude profiles for the polar (80°N and 80°S), mid-latitude (40°N and 40°S), and tropical (10°N and 10°S) regions, for each January and July. We did the same for the corresponding atmospheric pressure-versus-altitude profiles. As the COSPAR pressure data only reach from 20-120 km, the pressure data were smoothly extrapolated from 20 to 0 km using the slope of the 1976 U. S. Standard Atmosphere at a resolution of 5 km (obtained from Table 1 in Part 4 of <sup>ref. 94</sup>). From these data, we calculated the water partial pressure for each of the above profiles, and the vapour pressure of liquid water<sup>50</sup> using the temperature at each altitude. The definitions of the ratio of water partial pressure to liquid-water vapour pressure and relative humidity are equivalent. Calculations of the former, therefore, yield the corresponding relative-humidity profiles. In Figure 6a, we show the range of relative humidity for each of the three regions (polar, mid-latitude, tropical) using the maximum and minimum relative-humidity values of each of the four profiles of each region as the bounding values.

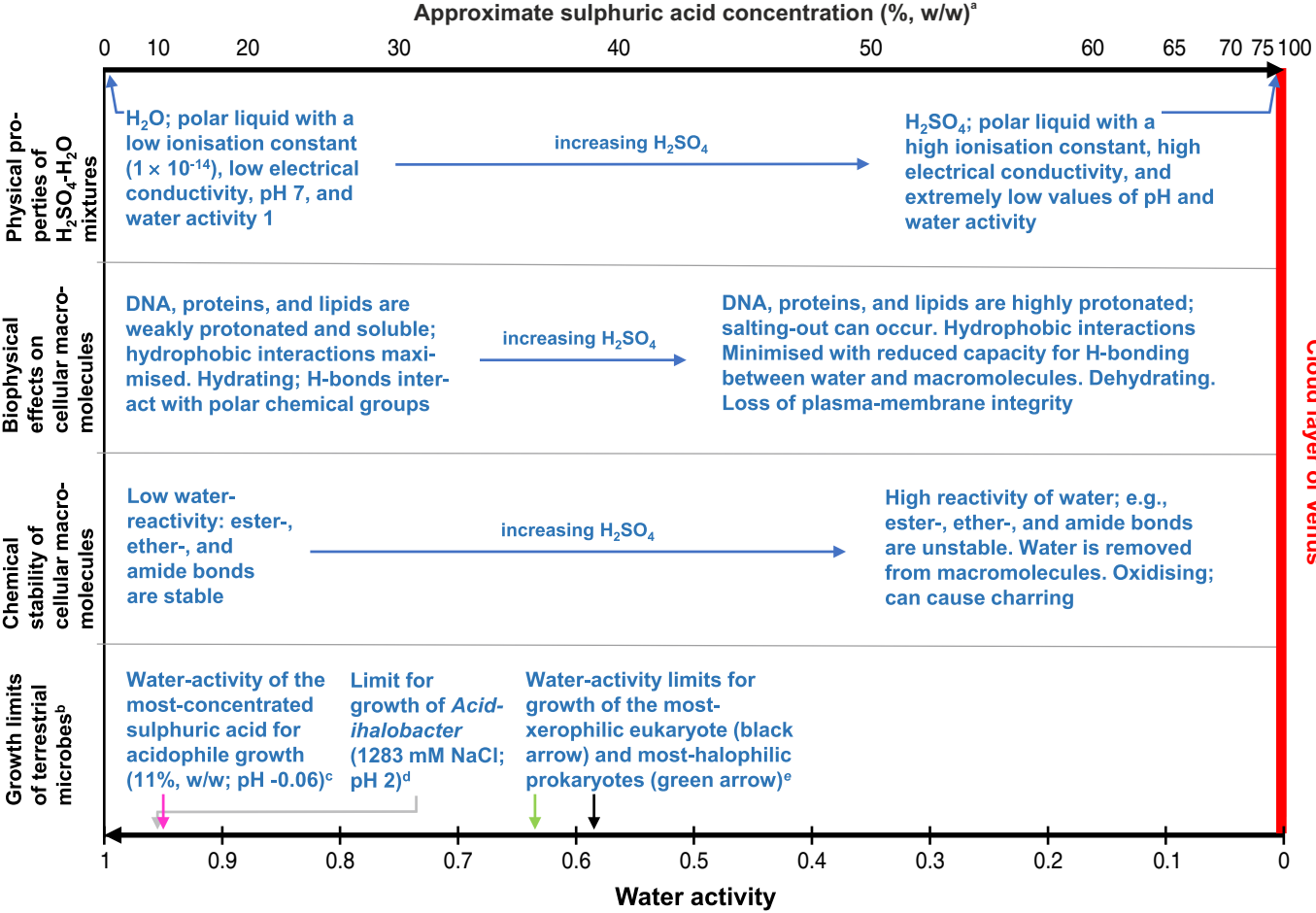
In Earth's troposphere, relative humidity is highly variable, both temporally and spatially. For our analysis of monthly average profiles, the largest variation occurs in the polar regions (Figure 6a). At higher altitudes, in the stratosphere and mesosphere, relative humidity is very low except for a local maximum in the summer polar mesosphere at about 85 km altitude, and a local maximum in the winter polar stratosphere at an altitude of about 25 km. Given that the stratosphere and mesosphere are very dry, clouds occur only during exceptionally cold periods in the polar regions. Typical conditions for cloud formation in Earth's atmosphere are detailed in Supplementary Table 3.

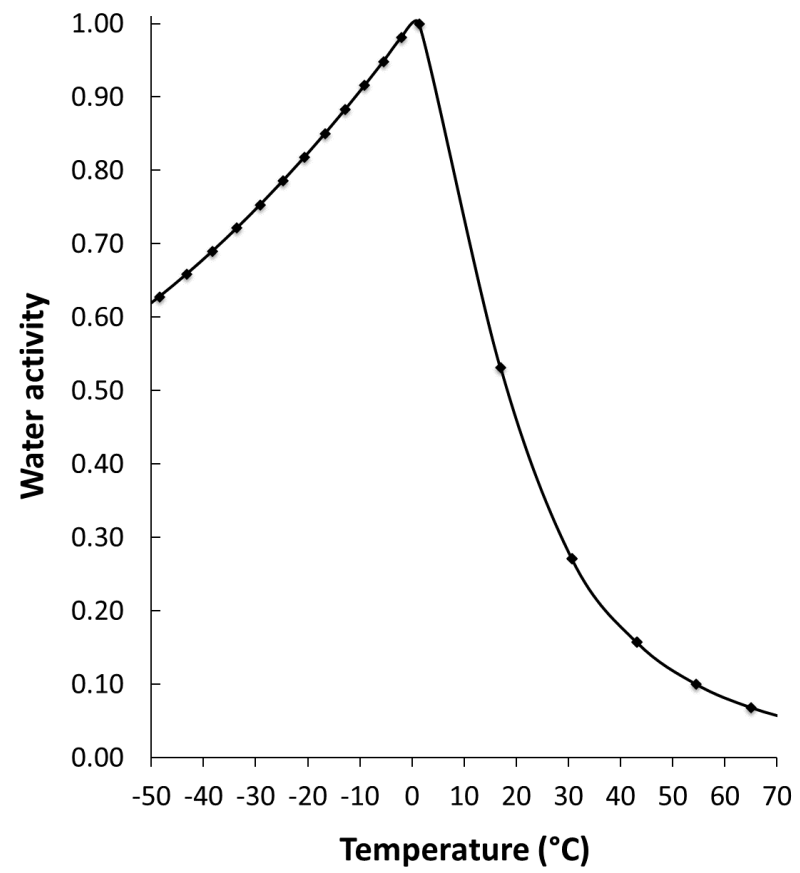
As in our analysis of Venus' clouds (above), the ambient relative humidity dictates the water activity of airborne microorganisms and liquid aerosol particles that may harbour lifeforms: under equilibrium conditions, which are often readily met in Earth's atmosphere, relative humidity and water activity are equivalent. Therefore, overall, the relative-humidity profiles in Figure 6 indicate that the water-activity values are on average too low for active life (i.e.,  $<0.585$ ) in the middle- and upper stratosphere and the mesosphere. The maximum water activity at altitudes of 30 and 80 km was about 0.003 and 0.006, respectively, and it was below  $10^{-4}$  to  $10^{-5}$  at altitudes in between. In contrast, high water-activity values occur frequently in the troposphere at nearly all altitudes, even for the latitudinal monthly averages used here. We note, however, that other factors (including ultra-violet radiation) impact microbial vitality and survival, and that these relative humidity profiles are more representative of average cloud-free conditions, and the actual variability is certainly much larger than the range indicated in Figure 6a. This is seen best by the average mid-latitudinal profiles (green area in Figure 6a), which are nearly always below a water activity of about 0.420 (42% relative humidity). Similarly, water-activity values are below about 0.60 (60% relative humidity) for the average tropical profiles at altitudes of about 10 km and below (red in Figure 6a). Such low values would imply that liquid water clouds, which require water-activity values of about 1 (100% relative humidity), would not be able to form in the mid-latitudes or the lower- and middle tropical troposphere. Clearly, this predication is not faithful to reality and indicates a dry bias when using monthly and latitudinal average profiles. In fact, in the tropical and mid-latitude regions of the lower and middle troposphere we would expect values of water activity of up to 1 (100% relative humidity). For these reasons, we used an alternative approach to estimate the typical water-activity values that occur within clouds (Figure 6b and Supporting 'Text for Figure 6b').

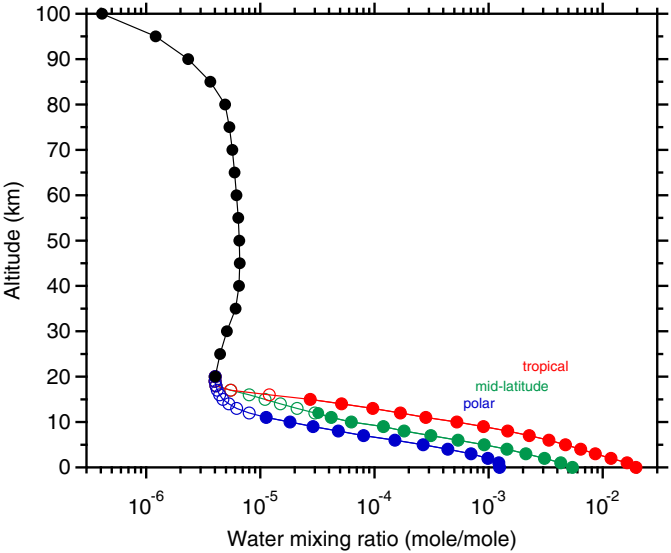




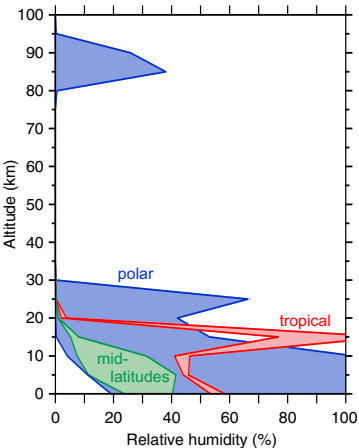








**(a)** average cloud-free



**(b)** typical in cloud

