Water activity in Venus' uninhabitable clouds and other planetary atmospheres

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

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

[2]*Faculty of Chemistry, Bielefeld University, 33615 Bielefeld, Germany.*

[3]*Centro de Astrobiologia (CSIC-INTA), 28850 Torrejón de Ardoz, Madrid, Spain.*

[4]*Department of Planetary Sciences, School of Geosciences, Meston Building, King's College, University of Aberdeen, Aberdeen, AB24 3UE, Scotland, UK.*

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

[6]*School of Natural Sciences and Centre for Environmental Biotechnology, Bangor University, Gwynedd, LL57 2UW, Wales, UK.*

[7]*Instituto Andaluz de Ciencias de la Tierra (UGR-CSIC), 18100 Armilla, Granada, Spain.*

[8]*Division of Chemistry, School of Pharmacy and Biomolecular Sciences, University of Brighton, Lewes Road, Brighton BN2 4GL, England, UK.*

[9]*Hillcourt Road, London SE22 0PE, England, UK.*

[10]*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 ≤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 ≤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.

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

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

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

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

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

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

Water activity and uninhabitability of Venus' clouds. There is no *a priori* reason to suppose that putative Venusian life would have the same biochemical basis as that on Earth. In the absence of any concrete proposal for an alternative biochemistry, however, several studies have considered whether living systems comparable to those on Earth might find viable niches on Venus, suggesting that this might be possible within the droplets of sulphuric acid clouds[5, 12,13].

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

Given that a permissive water activity is a prerequisite for active metabolism of terrestrial life-forms, we considered the water activity within Venus' clouds. We determined how sulphuric acid modifies water-vapour pressure of a liquid phase by converting water-vapour pressure data for H_2SO_4-H_2O mixtures[20] to water-activity values (Figure 1; data are provided in Supplementary Table 1). We observed a strong reduction of water activity even at modest sulphuric acid concentrations. We employed two independent thermodynamic models of sulphuric acid-water mixtures. The first model was from a study of Gmitro and Vermeulen[20] and is particularly well-suited for medium-to-high concentrations of (and up to pure) sulphuric acid over the entire temperature range of relevance to the current study. The second, the Extended Aerosol

Inorganics Model (E-AIM) by Clegg *et al*.[19] 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[20,21], 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[19] 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)[22], 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[23-26]. At the altitudes pertinent to biology (40 to 70 km; i.e., about +130 to -40ºC), 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[27,28] (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[20,21] (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*.[19] 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[29-31]. Given that Earth's entire functional biosphere spans only about 0.415 water-activity units, from 1 to 0.585 [ref. 32], 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') [5,29].

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[33], 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[33]. Furthermore, observations in Earth's stratospheric aerosol layer support this notion[30].

Seager *et al*.[13] 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[4]. 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[34]. At low water-activity, microbial cells adapt to retain their functionality by changing the composition of the plasma membrane; accumulating stress metabolites[35]; preferentially accumulating chaotropic substances at low temperatures to retain flexibility of their macromolecular systems[36]; 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[36-38].

In their analysis of cloud habitability, Seager *et al*.[13] 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 [ref. 39]. 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 *Acidihalobacter 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[40]. 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[41]. 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[42]. Here, we use the temperature-pressure profiles from this probe; the Galileo Atmospheric Entry Probe[43]. 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[44]. 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.*[45] 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 1.71×10^{-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[43]. 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_3) 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°C and -40°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[47]). Whereas clouds have been observed in the atmosphere of Mars, the temperatures are less than -73°C (200 K) regardless of altitude or location as determined by entry probes and global remote sensing[48,49]. 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°C is about 0.537, and then drops sharply with decreasing temperature (computed from vapour-pressure formulae of liquid water and ice in [ref. 50]), 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°C (215 K). We note that temperatures below this -58°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 O/H. For example, Kreidberg *et al*.[51] 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

267 temperature. For this reason, the lowest temperature considered is -59°C because below that
268 value, the water activity of ice is less than 0.585.
269
270 **Implications and Perspectives.** For a desiccated environment, whether a planetary atmos-
271 phere, surface or subsurface, the presence of liquid water does not necessarily indicate habita-
272 bility. Temperature must be permissive for cellular integrity and function, energy sources and
273 nutrients must be available and, critically, water activity must also be permissive for life. This is
274 illustrated by our case study of Venus' clouds where viable microbe-water relations are a key
275 prerequisite that – based on our knowledge of life on Earth – cannot be ignored. Indeed, it is on-
276 ly the two parameters of temperature and water activity that are considered determinants of hab-
277 itability for the purposes of planetary protection[4]. It has been suggested that the droplets of Ve-
278 nus' clouds can act as a protective environment for microorganisms[13], but we believe that cells
279 could not retain their integrity and/or functionality there due to the low water-activity and biophys-
280 ical and chemical effects of the highly concentrated sulphuric acid. Whereas we find the Greaves
281 *et al*.[5] report of phosphine to be highly intriguing, other studies refute this finding, for example [ref.
282 52,53,54]. Based on the current study, we must imagine a qualitatively new type of organism to in-
283 voke a plausible story about life in the Venus atmosphere, at least for life as we know it.
284 Based on the findings of the current study (in relation to water-activity and temperature),
285 the Jovian conditions make Jupiter's clouds currently the most-likely cloud formations in which
286 life could exist in our Solar System apart from those of Earth. A similar analysis would be of in-
287 terest for Saturn, Uranus, or Neptune, once entry-probe missions have been conducted. At pre-
288 sent, we lack empirical data for suitable exoplanet atmospheres. However, we mapped out an
289 approach whereby water-activity determinations can be made. It may be that such analyses form
290 a key part of assessments in future to identify exoplanets based on determinations of their habit-
291 ability. The James Webb Space Telescope (JWST) will be able to determine atmospheric pro-
292 files of temperature, pressure, and water abundance in exoplanet atmospheres[55], and these will
293 allow assessments of water activity in their atmospheres using our approach.
294 On Earth, life in the atmosphere has co-evolved with life in the oceans, on the surface,
295 and in the subsurface. Terrestrial microorganisms are known to influence atmosphere composi-
296 tion, hydrological cycle, and meteorology. For example, microbes produce and consume various
297 greenhouses gases; synthesise the stress metabolite dimethyl sulphide that volatilises and influ-
298 ences climate[56]; nucleate ice[57]; and drive cloud formation and precipitation. According to the
299 findings of the current study, the Earth's troposphere is for the most part biologically permissive,
300 the middle- and upper atmosphere become too dry for active life. Follow-on studies are also
301 needed to consider the type of intimate relationship that can occur between the atmosphere's
302 microbiome and other aspects of the planetary atmosphere, including climate and weather.
303 It should also be noted that on Earth (and to a lesser extent on Jupiter and possibly on
304 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').

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

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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_2SO_4-H_2O 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 H_2SO_4-H_2O 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[20,21] 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 [ref. 14]; 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 [ref. 18] (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 (-40ºC to +130ºC). The uncertainty in altitude is smaller than the size of the data-point markers. The uncertainty in water activity is ±30% of the value, as described in the text.

Figure 3. Schematic diagram to show the implications of the water-activity values of H_2SO_4-H_2O 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 H_2SO_4-H_2O 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. [4,14,18]). **a.** The approximate sulphuric acid concentrations at 25°C relating to the water activities given on the right were calculated using E-AIM[19] and Gmitro and Vermeulen[20,21] 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[19] - pink arrow; see Methods). For further information about H_2SO_4-H_2O 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[3] and the acidotolerant xerophile/halophile *A. penicillioides* can grow down to 0.585 water activity (black arrow) at high glycerol concentration[14]. 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.*[19]).

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[23]. A constant water-vapour mixing ratio is assumed to be set by the solar ratio of O/H[46]. 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[58] (solid blue, green, and red circles, respectively), which were then interpolated (open circles) to the average profile in the stratosphere and mesosphere[59] (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[60] 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[14] - for a water-vapour mixing ra-

tio set by the solar ratio of O/H. For values to the right of the curve, the values of water activity are less than the 0.585 water-activity limit while for values to the left of the curve the water activity is greater than this limit. The lowest temperature considered is -59°C; below that value the water activity of ice is less than 0.585.

Table 1. Water activity and sulphuric acid concentrations of droplets in the Venusian cloud layer[a].

Temperature (°C)[b]	Altitude above mean ground level (km)[b]	Pressure (bars)[b]	Relative humidity (%)[c]	Water activity[d] of cloud droplets	Sulphuric acid concentration of cloud droplets (%, w/w)[e]
-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

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

b. Altitude, temperature, and pressure values are from entry probe data with uncertainties of ±1 km, ±1K, ±5%, respectively[23].

c. Values were computed using the mixing ratio of water from observations as parameterised by Gao *et al.* [26]. Uncertainty, based on reported uncertainties in atmospheric profile, is ±30% of the value.

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

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

Methods

Water activity of H_2SO_4-H_2O 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 H_2SO_4-H_2O 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[20,21] 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[20,21], 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[50] [up to and including 0°C] and Wagner and Pruss[61] [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(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[20,21] 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(water-activity) : sulphuric acid-concentration data pairs.

The E-AIM[19] 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[62]. 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 H^+ activity coefficient, which was converted to the molality-based activity coefficient[63] and then the molality-based H^+ activity in solution, from which the molality-based pH (the negative decadal logarithm of H^+ activity according to IUPAC convention[64]) is derived. E-AIM[19] 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[19] 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[20,21] 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[19] (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_2SO_4-H_2O 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, ±30° [ref. 43]. These datasets report values for every kilometre in altitude and reported uncertainties in this profile are ±0.15 km for altitude, ±5 K for temperature, and ±5% for pressure[46]. Day to night temperature differences are only about ±5 K [ref. 43]. The water-vapour mixing ratio is taken from the parameterisation of Gao *et al*.[24] which is based on observations from Bertaux *et al*.[25] and the Venera 11, 13, and 14 missions[24].

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[50]. 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*.[26], we estimate to be ±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[26], 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[29]. The droplets are large enough (radius approximately 1 μm) that the reduction of water activity due to curvature in the droplet (the Kelvin effect) is negligible[65,66]. 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 $\alpha\, n\, 4\, \pi\, r^2\, (kT/2\, \pi\, m)^{1/2}$ [ref. 67]. Where α 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 μm which is smaller than, or comparable to the droplet size of about 1-μm radius.

Expressed in terms of the e-folding time, for the droplet to grow in size by a factor of e, we have $\tau^{-1} = r^{-1}dr/dt = r^{-1}\,\alpha\,(n/\rho)\,(mkT/2\pi)^{½}$, where ρ 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 ~ 1 atm, temperature ~ 0°C, and water-vapour concentration ~ 20 ppm. We obtain a value of T = 0.4 s, for α = 1. The mass accommodation, α, is typically assumed to be unity in models of Venus cloud physics[26]. However, Gardner *et al.*[68] reported a lower limit to α 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 τ can be compared to the time for a 1-μm radius droplet to settle 1 km which is the step size in our atmospheric model. The settling velocity of a 1-μm radius droplet at standard pressure is $\sim 10^{-3}$ 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.*[29] give an eddy coefficient, D, at 60 km of 4×10^4 cm^2 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 2.5×10^5 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 [ref. 29]).

H_2SO_4 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. [5,11,13,69,70]), 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[20,21] 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[19], 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[20,21] data, to within ±1% (w/w) sulphuric acid for temperatures between -40°C to 25°C. The formation of solids will not occur, so was suppressed in the model. If we assumed that there is an uncertainty of ±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°C, which is equivalent to a sulphuric acid concentration of about 1.2% (w/w) according to Clegg *et al*.[19]. Above 50°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°C is 0.745[71]); see Figure 1A of Lee *et al*.[72] and Figure 1 of Harrison *et al*.[73]. 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[14,32], is also acidotolerant (see below). At this water activity, differentiation and cell division were observed on a nutrient medium supplemented with 7.7 M glycerol, at pH 6.1 [ref.14]. 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[74]. The lowest water-activity at which proliferation of halophilic prokaryotes has been observed is 0.635, in a NaCl-dominated but $MgCl_2$-rich (bittern) brine[3]. 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[75]. 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 mM NaCl and pH 2 (adjusted by addition of concentrated sulphuric acid to the nutrient medium; 30°C) [ref. 76,77]. In these studies, the final concentration of sulphuric acid was not empirically determined. Therefore, we used E-AIM[19] to calculate the concentration of sulphuric acid which corresponds to a pH of 2 at 30°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 [ref. 78]), 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[76].

The 0.585 water-activity limit for *A. penicillioides* 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[20,21] and E-AIM[19], and a molality-based pH value of -1.55 according to E-AIM and IUPAC convention[64]. However, growth of *A. penicillioides* has only been recorded down to pH 3 (on a citric acid/Na_2PO_4-buffered nutrient medium) according to a study of germination over a range of pH (Supplementary Figure 2; [ref. 79]). 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.*[80]) 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_{max}) was set at 10; the pH below which no germ-tube growth occurs (pH_{min}) was provisionally set at 2; the pH at which that rate of germ-tube growth was optimal (pH_{opt}) was set at 6.5; and the optimum rate of germ-tube extension (μ_{opt}) was set at 0.0188 mm d^{-1} in order to obtain a curve (Rosso *et al.*[80]). 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 1.07×10^{-6}. 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[81].

$$\frac{\theta}{S(\theta)} \leq S(\hat{\theta})(1 + \frac{p}{n-p} F^{\alpha}_{p;n-p})$$

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.*[19]. 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[19] and Gmitro and Vermeulen[20,21] 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_2SO_4-H_2O 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_2SO_4-H_2O 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[82]. 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 [16,34,35];
- 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[83,84]. 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.*[19] 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 [refs. 4,16,34,85], 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[86]. 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[36], 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 ([ref. 87] and references therein;[88]). 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

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

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

941 The situation would be further complicated for sulphuric acid by the likely extreme proto-
942 nation of any protein-like macromolecules, and perhaps by the disruptive 'salting-out' effect ex-
943 erted on proteins by sulphate- and hydrogen sulphate ions, amongst other adverse effects (Fig-
944 ure 3). Might sulphuric acid itself act as a biophilic solvent, though? There seems no reason to
945 rule out this speculative possibility *per se*. Sulphuric acid is capable of forming hydrogen bonds
946 with water molecules, hydroxyl groups and carboxylic acids[92,93], and it is a polar molecule with a
947 dielectric constant comparable to that of water (around 100 and 80 respectively). But it cannot
948 be expected to mediate macromolecular interactions in the same way that water does. The role
949 of water's motions and fluctuations on biological macromolecules seems to depend for example
950 on the highly cooperative dynamics created by its three-dimensional hydrogen-bonding network.
951 So, there would then be little justification for extrapolating from the biochemistry of terrestrial or-
952 ganisms at all.

953

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

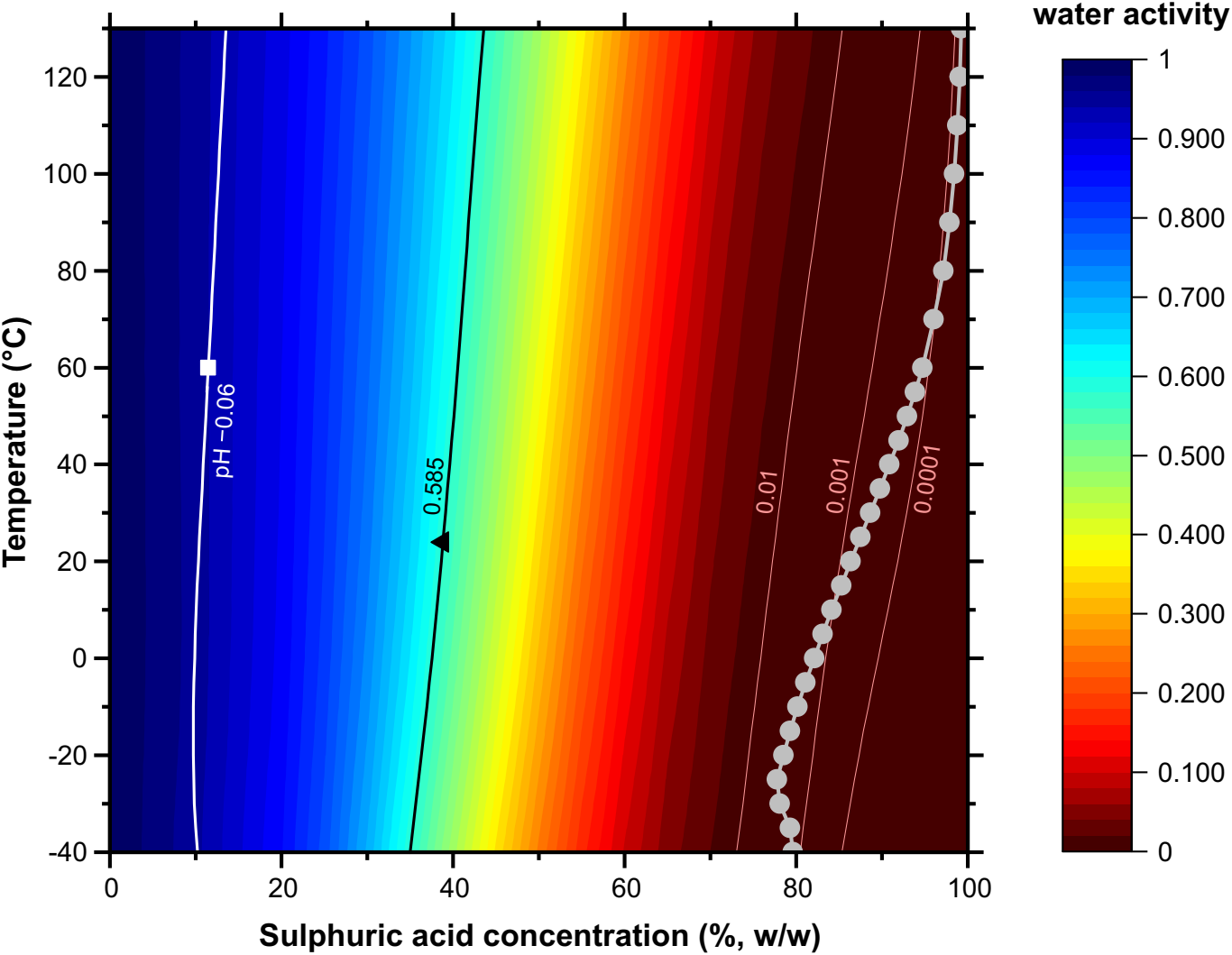
calculation indicates that ice is present, the water activity is equal to the ratio of the vapour pressure of ice to the vapour pressure of pure liquid water. This ratio is also how the water activity at Martian temperatures was computed. The method employed for Jupiter can be applied to exoplanets when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

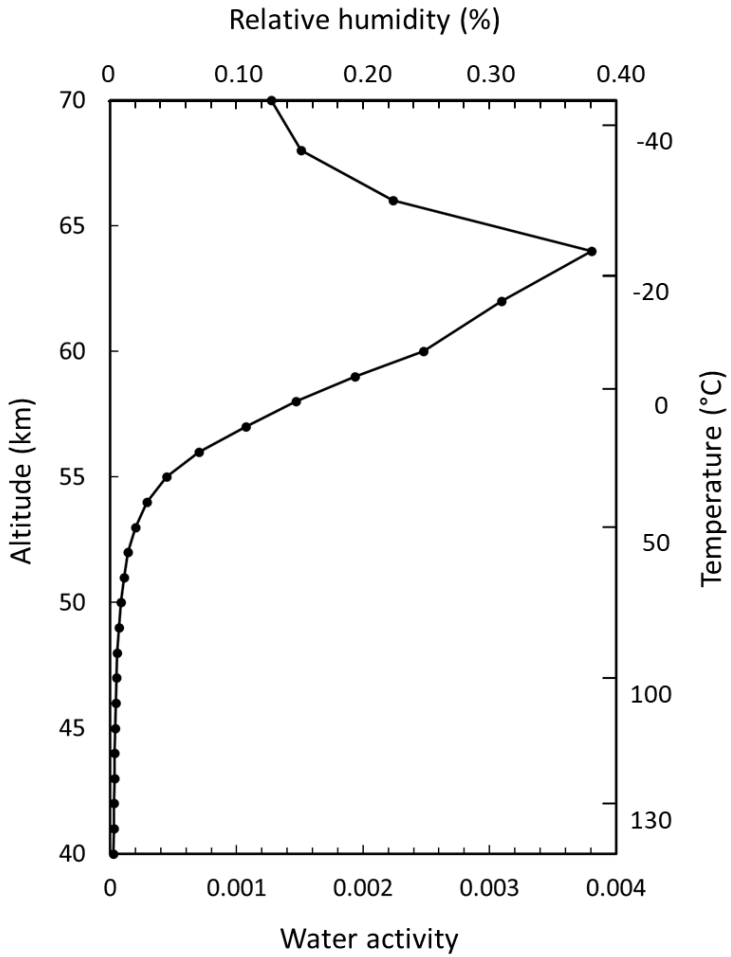
Analyses of water activity and habitability of Earth's clouds: A comparative case study. We applied our approach of computing the water activity from temperature and water-vapour abundance data also to Earth's atmosphere and clouds. For this purpose, we used the COSPAR International Reference Atmosphere[60] 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)[58] and for altitudes of ≥20 km, one average profile (solid black circles in Figure 5) which is based on data from Bohren and Clothiaux[59]. 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).

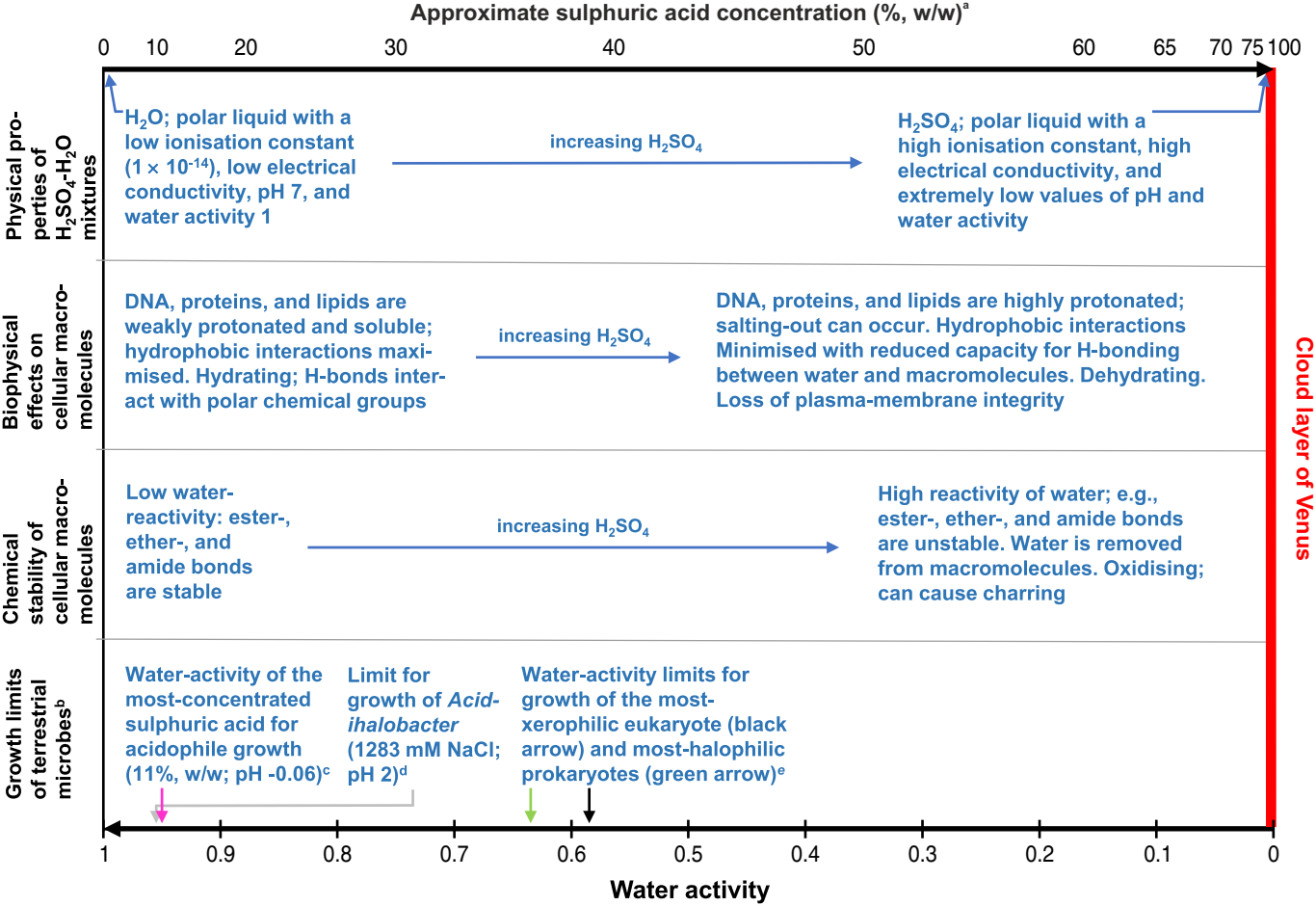
These water-vapour mixing ratios can then be used together with the COSPAR International Reference Atmosphere[60] average temperature- and pressure profiles to calculate relative humidity and water activity. For this purpose, we extracted temperature-versus-altitude profiles for the polar (80°N and 80°S), 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 [ref. 94]). From these data, we calculated the water partial pressure for each of the above profiles, and the vapour pressure of liquid water[50] 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.

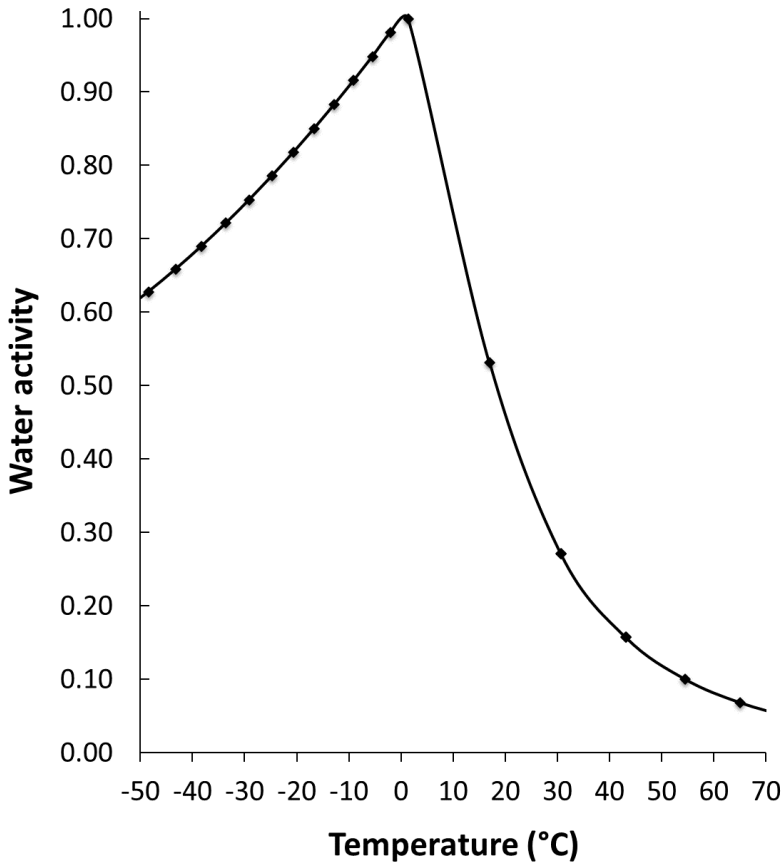
In Earth's troposphere, relative humidity is highly variable, both temporally and spatially. For our analysis of monthly average profiles, the largest variation occurs in the polar regions (Figure 6a). At higher altitudes, in the stratosphere and mesosphere, relative humidity is very low except for a 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.

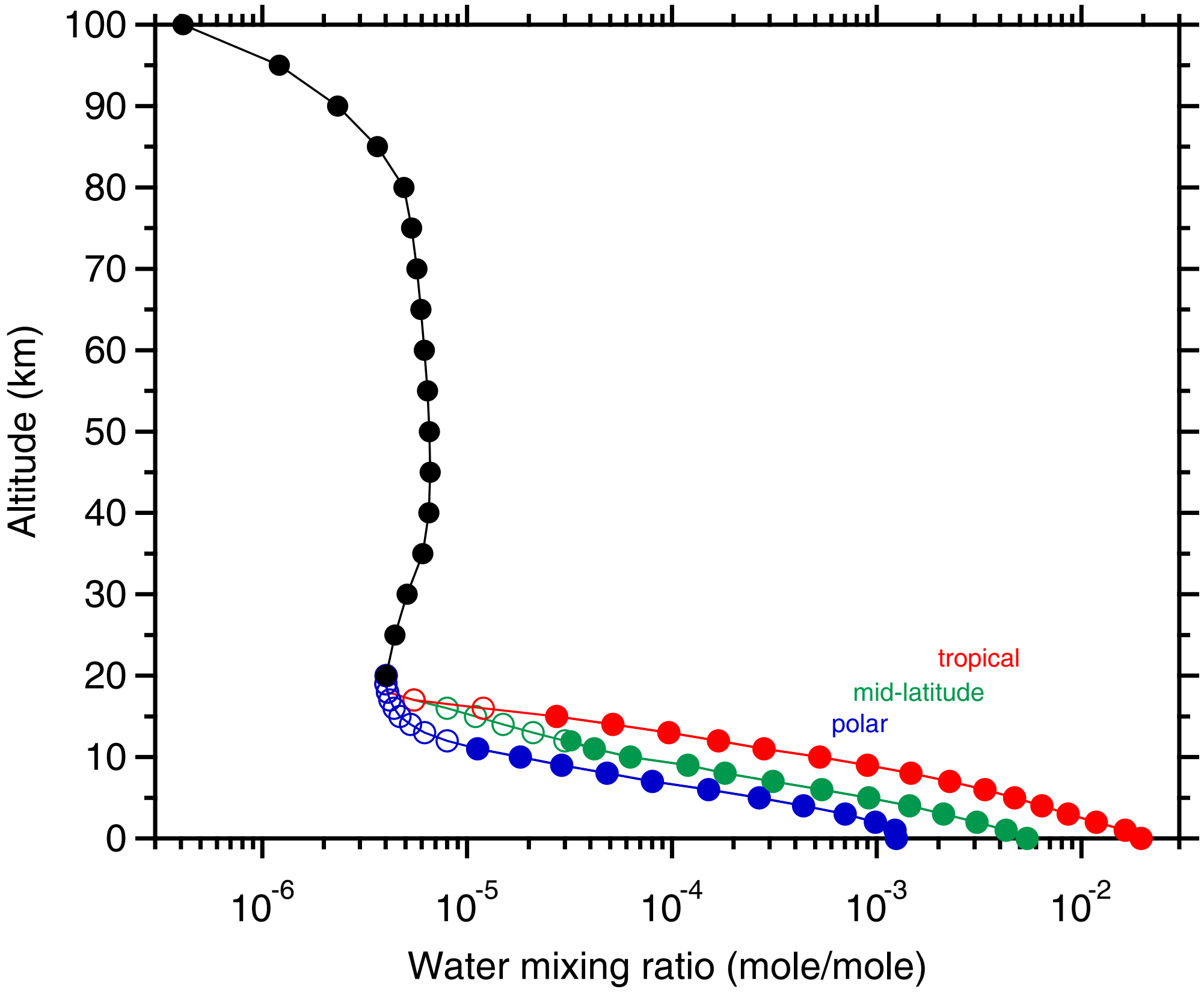
As in our analysis of Venus' clouds (above), the ambient relative humidity dictates the water activity of airborne microorganisms and liquid aerosol particles that may harbour lifeforms: under equilibrium conditions, which are often readily met in Earth's atmosphere, relative humidity and water activity 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').

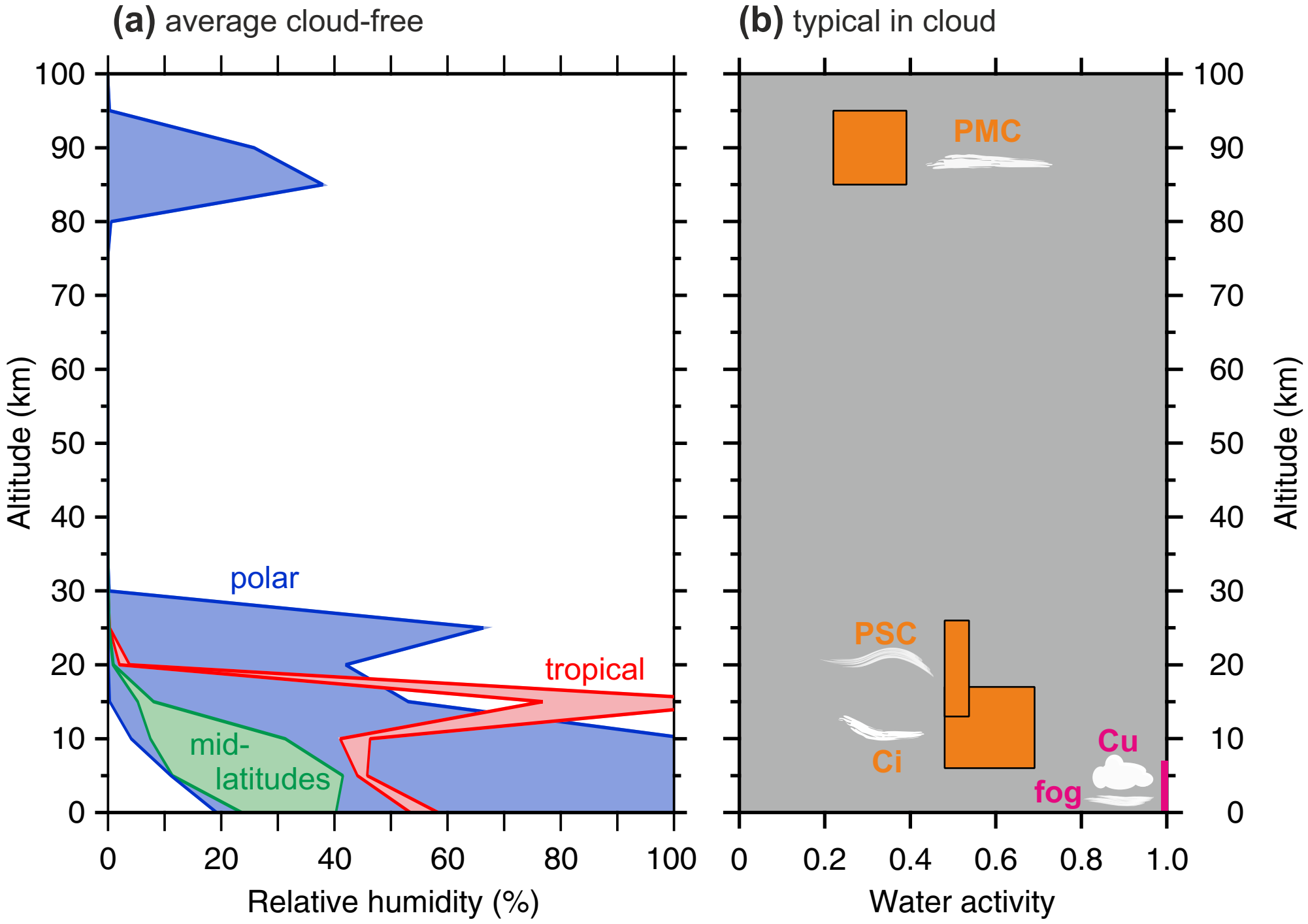

water activity
1
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0
Temperature (°C)
120
100
80
60
40
20
0
-20
-40
Sulphuric acid concentration (%, w/w)
0
20
40
60
80
100
pH −0.06
0.585
0.01
0.001
0.0001

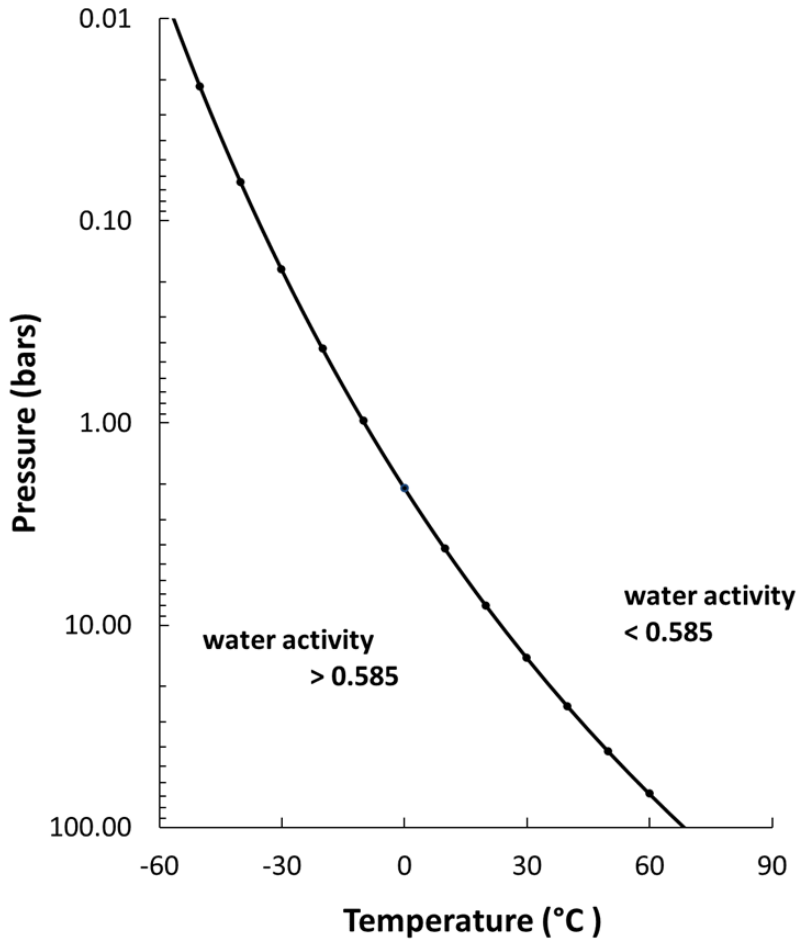

Relative humidity (%)
0
0.10
0.20
0.30
0.40
70
65
60
55
50
45
40
Altitude (km)
-40
-20
0
50
100
130
Temperature (°C)
0
0.001
0.002
0.003
0.004
Water activity


Approximate sulphuric acid concentration (%, w/w)[a]
0 10 20 30 40 50 60 65 70 75 100
Physical properties of H_2SO_4-H_2O mixtures
H_2O; polar liquid with a low ionisation constant (1×10^{-14}), low electrical conductivity, pH 7, and water activity 1
increasing H_2SO_4
H_2SO_4; polar liquid with a high ionisation constant, high electrical conductivity, and extremely low values of pH and water activity
Biophysical effects on cellular macromolecules
DNA, proteins, and lipids are weakly protonated and soluble; hydrophobic interactions maximised. Hydrating; H-bonds interact with polar chemical groups
increasing H_2SO_4
DNA, proteins, and lipids are highly protonated; salting-out can occur. Hydrophobic interactions Minimised with reduced capacity for H-bonding between water and macromolecules. Dehydrating. Loss of plasma-membrane integrity
Chemical stability of cellular macromolecules
Low water-reactivity: ester-, ether-, and amide bonds are stable
increasing H_2SO_4
High reactivity of water; e.g., ester-, ether-, and amide bonds are unstable. Water is removed from macromolecules. Oxidising; can cause charring
Growth limits of terrestrial microbes[b]
Water-activity of the most-concentrated sulphuric acid for acidophile growth (11%, w/w; pH -0.06)[c]
Limit for growth of *Acidihalobacter* (1283 mM NaCl; pH 2)[d]
Water-activity limits for growth of the most-xerophilic eukaryote (black arrow) and most-halophilic prokaryotes (green arrow)[e]
Cloud layer of Venus
1 0.9 0.8 0.7 0.6 0.5 0.4 0.3 0.2 0.1 0
Water activity


1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Water activity
-50 -40 -30 -20 -10 0 10 20 30 40 50 60 70
Temperature (°C)


Altitude (km)
100
90
80
70
60
50
40
30
20
10
0
tropical
mid-latitude
polar
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}
Water mixing ratio (mole/mole)


(a) average cloud-free
(b) typical in cloud
Altitude (km)
Relative humidity (%)
Water activity
polar
tropical
mid-
latitudes
PMC
PSC
Ci
Cu
fog
0
10
20
30
40
50
60
70
80
90
100
0
20
40
60
80
100
0
0.2
0.4
0.6
0.8
1.0


0.01
0.10
1.00
10.00
100.00
Pressure (bars)
-60
-30
0
30
60
90
Temperature (°C)
water activity
> 0.585
water activity
< 0.585