Removal mechanisms of dew via self-propulsion off the gecko skin

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Condensation resulting in the formation of water films or droplets is an unavoidable process on the cuticle or skin of many organisms. This process generally occurs under humid conditions when the temperature drops below the dew point. In this study, we have investigated dew conditions on the skin of the gecko Lucasium steindachneri. When condensation occurs, we show that small dew drops, as opposed to a thin film, form on the lizard’s scales. As the droplets grow in size and merge, they can undergo self-propulsion off the skin and in the process can be carried away a sufficient distance to freely engage with external forces. We show that factors such as gravity, wind and fog provide mechanisms to remove these small droplets off the gecko skin surface. The formation of small droplets and subsequent removal from the skin may aid in reducing microbial contact (e.g. bacteria, fungi) and limit conducive growth conditions under humid environments. As well as providing an inhospitable microclimate for microorganisms, the formation and removal of small droplets may also potentially aid in other areas such as reduction and cleaning of some surface contaminants consisting of single or multiple aggregates of particles.

1. Introduction

Geckos have received considerable interest, generally focusing on the adhesion properties of the small structures (setae) on their feet (typically Gekko gecko; [1–7]). The remaining regions of the lizard body have received very little attention in relation to specific functions which may be associated with the nano- and microstructuring [8,9]. This is somewhat surprising as geckos have interesting and intricate microstructuring on the skin regions. Foot microstructuring in geckos may also be evolutionarily linked to the body skin microstructuring (e.g. substructure of scales) [10].

One prominent feature of this group of lizards is the small hairs, typically called spines, spinules or microspinules spaced 0.2–0.7 μm apart and up to several micrometres in height [11,12]. A previous study has shown that these spines can be water-repellent and suggested that they may serve as a self-cleaning surface where rain may carry away contaminants [8]. Maintaining the surface of the gecko skin free from a water film may be beneficial for a number of reasons. For example, the growth of many microorganisms is enhanced by increased water availability and proliferation may result from such wetting conditions. Indeed, studies have shown that some lizards are susceptible to various external contaminants that can cause serious skin problems and diseases [13]. High humidity conditions and low temperatures have been shown to act as potential factors in the development of such reptile bacterial infections [14]. Thus, the wetting behaviour of the skin (the mechanical barrier and potential portal) plays an important role in particle and microbial resistance.
We have investigated a ground-dwelling gecko (*Lucasium steindachneri*) that lives in semi-arid habitats, where contact via rain is limited. For these geckos, rain may be totally absent between skin-shedding events; however, these lizards live in an environment in which humid air and low overnight temperatures can potentially result in condensed liquid water (dew). Thus, the behaviour of the condensate on the lizard skin is a possible source of water contamination and thus the focus of our study.

Recently, it has been shown on some natural and artificial superhydrophobic surfaces that when small condensing water droplets merge, as they grow in size, the combined single droplet is self-propelled off the surface [15–17]. This process arises from a change in surface energy from the transformation (coalescence) event [15,17]. Interestingly, this process has also been shown to aid in removal of small contaminating particles from surfaces and is thus a potential mechanism for self-cleaning [17]. To date, the only reported examples of the self-propulsion process on an animal surface has been on insect wings [17,18]. Obvious questions that arise from these studies include

1. To what extent does this self-propulsion process occur in nature?
2. Are surfaces with more intricate and complex hierarchical structures than simple insect cuticles used for such functionality?
3. Does the process aid in species survival?
4. Importantly, what specific mechanisms are used and what factors are involved in the process in total removal of water droplets from the surface (e.g. other environmental forces, habits, shape of organism)?

In this study, we describe the intricate topographical surface structure of the body of the gecko (*L. steindachneri*) and investigate the wetting formation under humid conditions and potential pathways by which condensates can be transported from the body’s surface.

2. Experimental procedure

2.1. Gecko capture and preparation

Box-patterned geckos (*L. steindachneri*) were captured at night by hand, from the Mingela Ranges (S 20°08’06” E 146°52’32”), Queensland (QLD), Australia. The Mingela Ranges are semi-arid, with a long-term (50 year) median rainfall of 493 mm, and 62.5 rain days per year on average [19]. Only healthy, adult lizards were returned to the laboratory, held in plastic containers with a heat source for thermoregulation, paper towel as substrate (changed weekly), a small tree branch and water ad libitum. They were fed domestic European crickets (*Acheta domestica*) three times a week. Geckos were allowed to shed twice, so that any oils were completely absent from their skin before experiments were conducted. Lizards were euthanized to ensure the skin’s surface was intact, and undamaged. Lizard skins were surgically separated by scalpel, cut into smaller sections (1–5 × 1–5 mm²) and attached to glass slides for droplet experiments.

2.2. Characterization of surface micro- and nanostructure

Scanning electron microscope (SEM) imaging was performed on a small section of lizard skin (approx. 3 × 5 mm²) mounted on an aluminium stub with carbon-impregnated double-sided adhesive, sputter coated with 7–10 nm of platinum, then imaged using a JEOL 7001 field emission SEM at 8 kV.

2.3. Condensation experiments

The sectioned skin samples were mounted on a cooled copper element maintained at a temperature below the dew point (16–20°C). The surface temperature of the skin was monitored with a dual-temperature meter HT-L13 with thermocouples (type K) and an infrared thermometer MT300. Typical conditions of dew formation were achieved in the laboratory with humidity of 75–85% and ambient temperatures of 24–27°C.

2.4. Droplet cleaning mechanism observations

Droplet formation and dynamics were captured using a Nikon (V1) digital camera (video mode at 400 and 1200 frames per second (fps), 640 × 240 and 320 × 120 pixel resolution, respectively, frame rates and acquisition times of 2.50 and 0.833 ms per frame and video play-back at 30 fps or 15 fps). Videos (4064) were taken observing condensation (duration ranged from 5 to 15 s), with another 336 videos observing small water droplets impacting with the gecko skin surface (dispensed and dispersed by spray bottle). The camera housing was attached to a Canon macro lens (EF-S 60 mm ultrasonic) for wide-field, low-magnification observations, whereas a custom-made optical microscope equipped with Olympus microscope lenses (MDFPlan) of 4× and 10× were used for higher magnifications (effectively up to approx. 300×). Contamination experiments were carried out using silica (KOB0 MSS-500/20—average diameter approx. 20 μm) and polymethylmethacrylate (Bangs Laboratories BB03N—mean diameter 83 μm) beads. These were seeded on the skin by distributing a thin coating (single and clumped) of particles onto a clean glass slide and then gently tapping the inverted slide over the skin.

3. Results and discussion

3.1. Topographical characterization of the gecko skin

Adult box patterned geckos *L. steindachneri* (figure 1a) typically achieve a length of approximately 55 mm with a mass of approximately 2.5 g and live in a range of areas including semi-arid regions and are typically ground-dwelling [20]. The optical images of the dorsal regions (figure 1a,b) show significant pigmentation which may serve as camouflage for the lizard. Previous studies have investigated the oberhautchen (outer thin layer) of numerous lizard skins and determined that they are composed of keratin [21–25]. X-ray photon electron spectroscopy data of the gecko skin in our study also suggest keratin is a component of the dorsal and abdominal regions where detailed scans showed binding energies of sulfur and nitrogen consistent with an organic environment.

The micro- and nanostructure on the dorsal and ventral scales was examined at higher resolutions using a SEM. On the dorsum, the scales were up to 200 μm in diameter with a similar centre-to-centre spacing and height, typically over 50 μm, and on the abdomen they were approximately 250 μm in diameter with a similar spacing (figure 1b,c). The areas between the scales comprise regions where the skin is heavily folded (figure 1d). The structuring on the scales at the micro- and nanolevel consists of spinules (hairs) and a basal layer. Scales have spinules with lengths of approximately 500 nm to
over 4 μm (figure 1e). The spinules are spherically capped with a radius of curvature less than 30 nm. The density of spinules is high: over 400 per 10 × 10 μm. The structuring below the spinules comprises a network of intersecting ribs forming a honeycomb-like basal layer (figure 1e). Lenticular sense organs are also visible on the scale edges (figure 1d). This micro- and nanostructuring is of sufficient density and roughness to indicate that the surface may have anti-wetting properties.

3.2. Wettability and dew formation under natural condensation conditions

The intricate patterning of the gecko spinule array topography facilitates a hydrophobic interaction with water. Static water contact angles confirm this and are generally within the range of 151–155° (figure 2a) on the dorsal and abdominal regions of the gecko, which is at the higher end of reported values on lizard skin [8,9] and superhydrophobic in nature. Hysteresis values were comparable to those of the lotus leaf. There are a number of theories to express the superhydrophobic condition, all of which have certain assumptions and limitations [26–30]. Cassie & Baxter [27] express the superhydrophobic state in terms of a number of interfaces: a liquid–air interface with the ambient environment surrounding the droplet and a surface under the droplet involving solid–air, solid–liquid and liquid–air interfaces. Equation (3.1) expresses the contact angle (θL) formed with a rough surface:

$$\cos \theta_L = f_1 \cos \theta_1 - f_2$$

(3.1)

where $f_1$ is the total area of solid under the drop per unit projected area under the drop, $f_2$ is defined in an analogous way of viewing the liquid–air component and $\theta_1$ is the contact angle on a smooth surface of the same material.

Equation (3.1) necessitates the surface to have the required roughness to allow air in topographically favoured regions such as troughs and surface depressions. Thus, topographies which increase the air–water interface and minimize the solid–liquid contact area may lead to higher contact angles. While the use of the ‘original’ Cassie–Baxter equation (equation (3.1)) is often preferable to other variations of the expression, it does require knowledge of the extent of penetration of the liquid into the depressions of the surface [30]. If the contact angle is known, however, then equation (3.1) can be used to predict as a first approximation the extent of penetration of the liquid into the troughs of a rough surface. From our measured geometric values and approximating the spinule terminus as a spherical cap (area = $2\pi rh$, where $h$ is the central distance from
the top of the sphere to the point at which liquid will invade) and $\theta_1$ as 115° and using a droplet invasion of 12.5 nm will calculate a predicted contact angle of approximately 175°. The experimentally determined value of 151–155° suggests that an area of approximately 40 times this value is required to bring the contact angle within the measured range. Thus, the droplet may invade to a greater extent. Inclined or slightly bent spinules near the apex may significantly increase the contact area with resting bodies on the surface. In addition, owing to the dome-shaped scale topography and folds on the skin surface, water droplets may interact with the spinule shafts to varied degrees. Indeed, the spinules may undergo bending (especially near the tips), which may also increase contact area. If we make a very basic approximation of the spinules as cylindrical spring structures and consider only the narrower end regions of the tapered shafts as the structural elements, then we can view a tip displacement by modification of a cantilever bending equation [31]

$$D_{sp} = \frac{4L^3F_{\text{drop}}}{3\pi N_{sp} R_{s}^4 E}$$  \hspace{1cm} (3.2)

where $L$ is the length (400 nm used in our case where a significant fraction of the bending will presumably occur), $E$ is the elastic modulus of the material (1.5 GPa), $F_{\text{drop}}$ is the total droplet force (a value of the radius, $R_{s}$, of 30 nm is used, reflecting the lower region of the cylindrical element (spinule)). For a water droplet of 2 mm diameter (approx. 4 $\mu$m) resting on the skin and assuming an equally distributed force contact on the end of around 100,000 spinules (based on spinule density and droplet contact area determined by microscopic imaging directly above the droplet), a deflection of over 5 nm would take place for each individual spinule near the tip.

It should be noted that the above description makes a number of assumptions and ignores numerous other parameters/factors that may cause the deviation of the contact angle from predictions, such as the non-homogeneity of the surface, defects, chemical variations and the natural bending itself as it appears in figure 1c. Nevertheless, the above-mentioned illustrative example provides some informative predictions for water interactions with surface projections of the scale as exhibited by the gecko and presents a useful comparative behaviour with smaller droplet interactions and other organisms.

The spinule topography on the gecko is different from the structure found on many other lizards [32–35]. Some other organisms, however, do have a dense spinule (hair) array...
3.3. Self-propulsion of condensing droplets from coalescence

To assess whether naturally occurring condensed droplets, such as those shown in figure 2, when merging will form larger droplets and remain pinned to the skin’s surface, or alternatively be self-propelled off the surface from changes in energy during coalescence, we conducted condensation experiments. By cooling the gecko skin below the dew point, surface condensation was induced forming droplets in the same size range as those shown in figure 2h–d. As they grew, some merged and then self-propelled from the skin surface. Figure 2r shows a snapshot in time of micro-droplets propelling autonomously off the skin (see the accompanying electronic supplementary material, movie S1). The fully merged propelled droplets were typically approximately 40–120 μm in diameter.

A recent paper [16] has shown that on a fabricated surface the subcooling value increases (termed ‘supersaturation’ by the authors) the micro/nanostructures fail to maintain the jumping-induced removal mechanism owing to an increase in the number of nucleation sites. This leads to ‘flooding’ and a loss of superhydrophobicity and the formation of highly pinned Wenzel droplet morphologies. In our case, humidity and temperature changes, while potentially large, generally occur over a relatively long period of time in the natural environment of the gecko, and thus flooding seems unlikely to occur. Observation of droplet numbers as shown in figure 2 supports this premise; however, how the gecko structuring performs in relation to fabricated surfaces in this area is of interest and worthy of exploration in future studies.

To investigate the range of droplet sizes involved, videos at higher magnification were collected of self-propulsion on both the dorsal and abdominal skin of the gecko. A sequence of images from the abdominal region of the gecko skin where droplets of varying size (from 10 μm to over 80 μm in diameter) merge and are self-propelled from the surface is seen in figure 2f (see also electronic supplementary material, movie S2).

This mechanism demonstrates that small water droplets can be projected from the skin surface with no external forces. If the lizard, however, is orientated in a horizontal position with respect to gravity as demonstrated in figure 2c, then the self-propelled droplet may eventually return to the skin under the influence of gravity. It may remain there if no other forces are acting on it and the droplet propulsion was orthogonal (normal) to the overall plane of the lizard’s skin. However, the ultimate destination of propelled droplets will depend on numerous factors (e.g. lizards in their natural environment will adopt several orientations and will also be exposed to other environmental influences and forces). With this in mind, we explored a number of skin surface orientations and observed the overall dynamics of the droplet-propelled process in different situations. We have identified a number of mechanisms that may aid in complete transfer of droplets from the surface of the skin.

3.3.1. Direct self-propulsion of droplets off the skin surface via the droplet merging process (single jumping event)

For illustrative purposes, the droplet propulsion process can be viewed in a very simplified manner by considering changes in the droplet surface energies. If we consider the case where two small water droplets (not necessarily of equal size) coalesce on a superhydrophobic surface to form a larger droplet, the maximum height, $H_{\text{max}}$, that can be attained by a droplet can be determined by integrating the velocity of a droplet over its time-of-flight resulting in equation (3.3):
duced when coalescence occurs with droplets of dissimilar sizes. The droplet size distribution from condensation, such as those seen in figure 2, ensures droplets of different sizes will coalesce frequently, although droplets of similar sizes will also merge (as those seen in figure 2c). Droplets exceeding the dimensions of the lizard scales (larger than 200 μm) are rare (figure 2c), thus droplets smaller than this will most often be involved in the self-propulsion process. This size range of droplets upon coalescence will also overcome adhesional forces from the surface, and, once free, these smaller droplets are less influenced by gravitational forces than larger droplets.

Equation (3.3) predicts a maximum height approaching 20 mm; however, the majority of droplets were propelled 0.5–2 mm (figure 2e and electronic supplementary material, movie S1). A more comprehensive view of the propulsion process has been presented by Feng et al. [42]. They view the initial total kinetic energy of the coalesced droplet as $E_{tk} = \Delta E_s - E_{vis} - E_h - E_w - E_{cab}$ where $E_{tk}$ is the initial total kinetic energy of the coalesced droplet, $\Delta E_s$ is the surface energy released by the droplet coalescence, $E_{vis}$ is the viscous dissipation in the droplet, $E_h$ is the gravitational energy change during droplet coalescence, and $E_w$ is the work of adhesion. $E_{cab}$ is the energy consumed overcoming the contact angle hysteresis.

The authors state that approximately 25% of the energy released by the droplet coalescence is converted to the effective kinetic energy in the vertical motion of the coalesced droplet jumping from the surface. Thus, the higher propelled heights predicted in equation (3.3) can be attributed to the omission of other factors such as viscous dissipation. The droplet propulsion process was also measured at 1200 fps, allowing droplet speed to be measured accurately. The typical initial velocities of droplets were below 0.4 m s$^{-1}$.

The merging of two or more droplets can cause self-propulsion off a surface (electronic supplementary material, movies S1–S2). If the gecko is orientated in a vertical position, then the single propulsion of a droplet can result in complete removal from the surface. It is evident that if the gecko is inclined at near-vertical angles (approaching 90°), then propelled droplets will be able to traverse large distances and are more likely to be totally removed from the surface (see electronic supplementary material, figure S1 and movie S3—surface inclined at approx. 70°). If the lizard is orientated in a horizontal or near-horizontal stance, then the underlying body and side regions of the gecko will also be amenable to this mechanism. The small size of the gecko and the rounded cross-sectional profile of the lizard body will also facilitate complete removal via a single propulsion of a water droplet. Once droplets are self-propelled from the skin in these situations gravity can assist in complete removal of the droplets. Thus, no other external force is required for droplet removal. As the distance that a droplet can be propelled is significant in relation to the smallest lateral dimensions of this lizard then this mechanism can, in principle, remove droplets from anywhere on the surface of the skin.

3.3.2. Self-propelled impacting droplets which laterally sweep the surface and facilitate removal of surface-bound condensation—removal facilitated by sufficiently high kinetic energy of impacting droplets

Merging droplets are typically propelled in near-vertical directions in relation to the skin surface (for example, electronic supplementary material, movie S1). Some proportion of droplets, however, is propelled at angles facilitating motion along the plane of the skin’s surface rather than directly out of plane. This lateral sweeping of droplets across the surface is often facilitated by the coalescence of multiple droplets of varying sizes (figure 3e and electronic supplementary material, movie S4). This may potentially be aided by the skin topography where droplets that merge are initially resting on scales and thus are on an inclined surface (figure 2f). Sufficiently large droplets merging can be driven along the surface with sufficient momentum to remove themselves from the surface and in the process collect smaller droplets along the way as shown in figure 3a,b (see also electronic supplementary material, movie S5). The process may be viewed as an extended form of the lotus effect (impacting and rolling droplets), where the initiating step is via self-propulsion(s) of condensation as opposed to a falling or rolling droplet from rain. Thus, the removal process may take the form of transformation of the
self-propulsion process to that resembling a rolling process along the surface as in the lotus effect.

3.3.3. Impacting droplets facilitate self-propulsion off the skin surface (impacting droplets from fog and small self-propelled droplets)

The situation where small falling water droplets impact stationary surface-bound droplets is shown in figure 4a,b. A falling droplet of 20 μm diameter with a velocity of 0.013 m s$^{-1}$ impacting a stationary surface-bound droplet of approximately 70 μm is illustrated in figure 4a and also in the electronic supplementary material, movie S6. Figure 4b shows a very small droplet (highlighted by the arrow) travelling at a slow velocity, making contact with two stationary surface droplets of approximately 100 and 70 μm in close proximity to each other, resulting in self-propulsion off the surface (see also electronic supplementary material, movie S7).

Fog conditions occur regularly in areas inhabited by this particular gecko (observation from collection and weather data for regions of habitat (Bureau of Meteorology [19])) and often coincide when high humidity conditions take place. Fog as well as previously self-propelled droplets may collide with the skin and merge with existing dew droplets, resulting in a newly merged droplet self-propelling off the surface. The process may be aided by kinetic energy of the impacting mobile droplet; however, for slow moving small droplets the surface energy changes are the primary driving force for propulsion of the final droplet. The change in surface energy from coalescence in figure 4a results in a self-propelled droplet approaching 0.1 m s$^{-1}$. Small, mobile impacting droplets may also facilitate self-propulsion by adding to the volume of stationary droplets forcing them to merge with neighbouring droplets (figure 4b and electronic supplementary material, movie S7). Propulsion arising from small impacting droplets may also be responsible for future coalescence and subsequent propulsion events.
3.3.4. Wind-assisted removal of self-propelled droplets
A significant population of propelled droplets have sufficient momentum to be transported outside the skin–air boundary layer (figure 2e), allowing them to be exposed to external environmental forces (such as light breezes). Importantly, the self-propulsion releases the droplet from the surface–air interface and associated adhesive forces and exposes it to forces that could potentially transport the drop significant distances away. As the droplets are extremely small, their mass will be sufficiently low that very slight external wind forces are able to fully remove the droplet from the skin once they have self-propelled above the surface. For a 50 μm diameter water condensate, a wind force of less than 1 nN is required to oppose the gravitational force. For a 200 μm droplet, the force increases to approximately 40 nN. Electronic supplementary material, movie S8, illustrates the susceptibility of droplets to removal if exposed to light breezes over the surface. Droplets can easily be transported several centimetres away from the point of origin. Wind forces may also initiate self-propulsion of droplets by transporting small droplets laterally on the surface forcing coalescence.

3.3.5. Other potential benefits of self-propulsion of condensed droplets
While the formation of high-contact-angle small micrometre-sized droplets and subsequent self-propulsion provides a landscape under dew conditions which can potentially limit the growth of microorganisms such as bacteria and fungi on the lizard skin, does the phenomenon offer any other possible attributes for the organism? It should be noted that the self-propulsion of water droplets on artificial superhydrophobic surfaces has shown to be a way of controlling heat transfer [16] and this may be relevant as some water-repellent reptile surfaces may contribute to thermoregulation.

The self-propelled droplet-assisted mechanisms as seen in figure 5 may also possibly aid in removal of some contaminants from the gecko skin. We have made a preliminary investigation to see if contaminants (e.g. silicon and polymethylmethacrylate...
particles) could be removed by condensation and self-propulsion processes. Figure 6a shows that a small silica particle encapsulated in a droplet can be removed via the droplet propulsion mechanism. Figure 6b shows groups of droplets (two and three droplets which house silica beads) demonstrating self-prolusion.

The gecko skin surface was also seeded with polymethylmethacrylate beads (PMMA—mean diameter of 83 μm with a contact angle of 60°) and exposed to condensation conditions. Large particles and particle clumps were transported along the surface with only small amounts of condensed water surrounding them (electronic supplementary material, movie S9). Particle clumps rearrange owing to meniscus forces, and the energy was released in the form of jumping conglomerates of particles (figure 7a,b and electronic supplementary material, movie S10).

While a previous study has shown a single jump of hydrophilic glass beads from a superhydrophobic wing surface [17], we have found that small clumps of these less hydrophilic PMMA particles (in heavily contaminated regions) tended to undergo sequential aggregation with a series of self-propulsions and can be finally propelled along or off the surface. The aggregate seen in figure 7b makes intermittent contact with the surface during the propulsion event by bouncing across the surface. Interestingly, PMMA clumps comprising only a few particles can be propelled from the skin surface (figure 7a). As well as nucleation occurring on the skin surface, figure 7a demonstrates that nucleation of droplets can occur on individual particles. It is apparent from figure 7a that only small condensed water drops or films relative to the particle size can facilitate self-propulsion off the surface. The lateral sweeping off of particles/droplets along the plane of the skin surface is shown in figure 7c (see also electronic supplementary material, movie S11). In this case, multiple droplets/clumps containing numerous particles can be transported along the surface leaving the skin free from contamination.

A few recent studies have investigated self-cleaning of the gecko foot [43,44] and suggested that adhesive forces attracting a dirt particle to the substrate and hyperextension are two significant factors for cleaning. Of note in our study are the similarities as well as the significant differences in foot structuring and spine structure (radius of curvature, multiple projections, etc.; see visual comparison in electronic supplementary material, figure S2).

4. Conclusion

The dense array of spinules on the gecko skin provides an architecture suited for removal of large water droplets. It also provides an ideal construction for the self-propulsion of small condensed droplets. Dew on some surfaces may promote the growth of pathogens such as fungi and bacteria and may enable spores to develop. In humid environments, this temporary water formation or thin film may result in proliferation resulting in larger populations of microorganisms. As the dew on the superhydrophobic gecko skin is a temporary phenomenon (constant self-propulsion of droplets and small-sized droplets leading to quick evaporation in sunlight/heated
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References

7. Bhushan B. 2009 Biomimetics: lessons from cleaning by these mechanisms, which have the potential to carry away small contaminants. Of particular interest is the removal of just a few particles with minimal water/droplet coverage and expansive cleaning from lateral mobile droplets brushing the surface clean. While we have identified removal mechanisms, the efficiency of cleaning the surface based on droplet and condensate processes for removing particles (e.g. minor contamination of biological matter, bacteria, soil particles) requires further investigation in subsequent studies.


