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PERSPECTIVE

# Is Plasma Activated Water Really Magical? A Reflection on the Phenomenon

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**Abstract**

A reflection on the phenomenon of plasma-activated water (PAW), its brief history and properties. PAW arises from the accumulation of reactive plasma products (mainly $H_2O_2$, $NO_2^-$, $NO_3^-$, $O_3$ and sometimes HNO, ONOOH) in water and has many interesting and beneficial properties on both living and non-living biological objects. It has attracted considerable attention in the last 15 years and raises the question whether it might not be simpler to prepare it artificially (APAW) directly by mixing chemical compounds. There are several papers which have compared the effects of PAW with APAW and conclude that there is probably no significant difference. In this paper, we conclude that the preparation of PAW is several times more expensive than that of APAW. However, we also note that there may be specific situations in which the production of PAW could be advantageous, such as its efficient role in storing energy in the form of nitrate ions, which can serve as a nutritional source for plants.

**Keywords** Artificial plasma activated water · Disinfection effects · Seeds germination · Plant growth · Fertilizer · Hydrogen peroxide

## Introduction

The microbicidal effects of non-thermal plasma have been studied for over 20 years and the research to explore this phenomenon is still ongoing. However, it has been also found that to achieve these effects, it is not necessary to apply plasma directly to the suspension of microorganisms, but these effects are preserved to a lesser extent in the form of plasma-activated water.

This phenomenon was probably first described in the work of Kamgang-Youbi et al. [1], where water was activated by gliding arc discharge, and its bactericidal effects were

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studied on *Hafnia alvei*. Unfortunately, this and subsequent work by the author [2] were published primary in biological journals, and therefore went unnoticed by the plasma physics community for several years. Perhaps this is why, as has occured many times before, the phenomenon was subsequently rediscovered by other research groups. Probably the first independent reference is our little-known paper [3], where this phenomenon was observed on *Escherichia coli* and *Staphylococcus epidermidis* after treatment with water activated by corona discharge. Subsequent studies by [4] using DBD and [5] using transient spark discharge for water activation followed and reported the effect also on *E. coli*. In the aftermath, papers that gained wider attention began to refer to this remarkable phenomenon by names such as 'water of dead' [6], the bizarre term as 'plasma acid' [7], 'plasma acidic water' [8], or 'plasma treated water' [2, 5, 9]. The term 'plasma activated water' (PAW) was reintroduced in the paper [10] and has gradually supplanted other established names. However, this designation is somewhat misleading, as no actual plasma activation of water occurs in this context. Unfortunately, a more appropriate term, plasma treated water, has not yet been widely adopted. Therefore, we will continue to use the established term PAW.

Since then, a substantial number of papers have been published on PAW in various fields of research and development related to living organisms. Numerous review articles have enthusiastically highlighted the amazing phenomenon of PAW. The primary objective of this text is not to offer yet another glorifying review, but rather to critically assess this enthusiasm. A similar concept regarding the assessment of PAW in the medical field was previously presented in paper [11]. Here, we would like to follow up on this work and bring a different PAW perspective focused on plants and address the inevitable question: "How is this possible?" If the composition of PAW is known, it should be much easier and more cost-effective to produce it artificially by mixing the appropriate chemicals. Unfortunately, studies comparing the effects of PAW with those of artificially prepared PAW (APAW) are scarce in the otherwise rich literature on PAW, which can be considered as a shortcoming in the design of many planned researches. If comparisons demonstrate that PAW and APAW have equivalent effects, it would be feasible to use the significantly cheaper and simpler APAW. Conversely, if PAW is found to be more effective than APAW (or vice versa), an important question arises regarding the underlying causes of this difference. Both scenarios should prompt a new direction in scientific research.

The aim of this paper is depicted in Fig. 1, where the main principles of PAW and APAW preparation are outlined for better illustration. It is important to note that the flowchart of the processes leading to PAW generation is very simplified.

## Amazing Effects of PAW

As previously mentioned, numerous studies highlight the remarkable potential of PAW in various fields related to biology.

It has been extensively investigated in recent years for its disinfection effects and preservation of food, including fresh fruits, e.g. [12–15], fresh vegetables, e.g. [16–19], or fresh sprouts, e.g. mung bean sprouts [20]. Furthermore, the disinfection of food contact materials [21] and other surfaces, including stainless steel and polyethylene [21], has also been reported.

In agriculture, numerous studies have reported the effects of disinfection on seeds, the positive influence on seeds germination and plant growth, and the PAW as an alternative to chemical fertilizers [22–25]. These improvements were demonstrated e.g. for lettuce

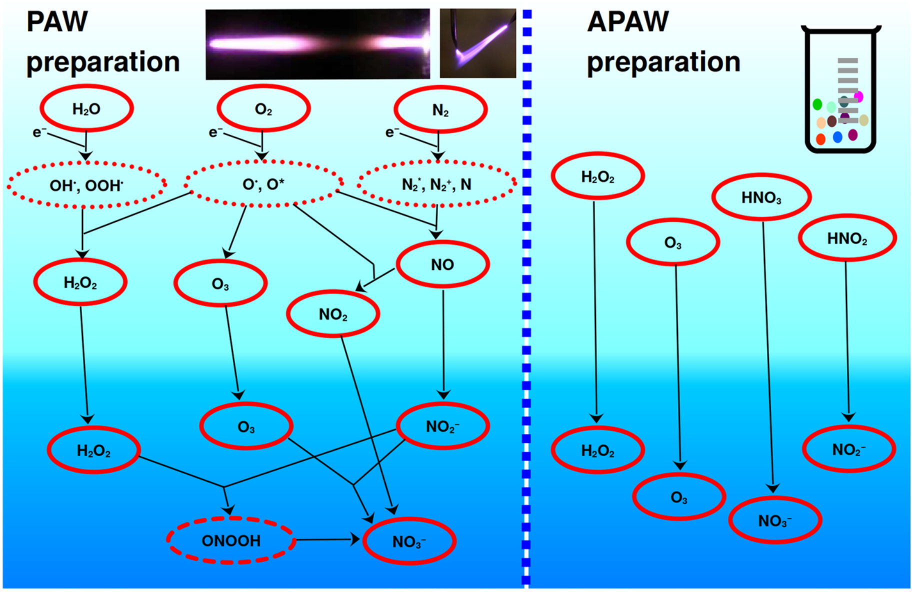

**Fig. 1** The very simplified main principles for preparation of PAW and APAW

(*Lactuca sativa*) [24], rice (Oryza sativa) [26], radish (Raphanus sativus), fenugreek (*Trigonella foenum-graecum*) pea (*Pisum sativum*) [27], and mung bean (*Vigna radiata*) [28]. More complex study on Barley (*Hordeum vulgare*) also showed the reduced levels of deoxynivalenol, a secondary metabolite produced by phytopathogenic fungi, and maintaining its quality [29]. Other study [23] on black gram (*Vigna mungo*) seeds also resulted in significant improvements in various agronomic traits, antioxidant enzyme regulation, and stimulated germination and growth. A review by [30] considered not only the effects of PAW on plant germination and growth but also its potential use in plant disease control and seed decontamination. Future research of [31] discusses and analyses the impact on treated plants, enabling the practical application of this technology.

Additionally, there is considerable interest in the effective and innovative substitution of synthetic nitrite in the manufacturing of cured meats [32, 33]. Research related to medicine has also been published regarding the use of PAW for treating skin wounds, e.g. in mice [34], and for the selective treatment of cancer cells, e.g. [35].

## Chemical Composition of PAW

Because of the listed interesting effects of PAW, the question of its composition is important. Many analytical studies have focused on it, including investigations into plasmochemical processes and the substances generated. It is accepted that the burning of the discharge produces various active particles in the plasma, which accumulate in the water, resulting in PAW. Because to prepare the PAW, the discharges are burned in an atmosphere with at least partial access to air, the main components playing roles are oxygen and nitrogen. Details can be found e.g. in the following notable examples including pilot articles such as [10, 36–42]. Additionally, an extensive review directly addressing PAW chemistry is provided by [43]. Many other review papers have also discussed the generation and composition of PAW, as well as its potential applications, such as [25, 44–49].

In agricultural applications, there will certainly be a delay between the preparation of PAW and its application, therefore, only long-lived reactive species are relevant [50]. The results indicate that the stable components capable of persisting in PAW for more than several seconds include hydrogen peroxide ($H_2O_2$), nitrite ($NO_2^-$), nitrate ($NO_3^-$), and ozone ($O_3$), with their proportions varying based on the conditions under which PAW is generated. Besides plasma-activated water, some other plasma-activated liquids (saline or nutrient solutions) are also used in agriculture, but the reactive particles considered are again only hydrogen peroxide, nitrite, and nitrate [51].

PAW often exhibits an acidic pH due to the presence of nitrite ($NO_2^-$) and nitrate ($NO_3^-$) ions. The lower pH level typically increases the stability of $H_2O_2$, which contributes to its oxidative strength and to PAW's biocidal and growth-promoting properties. Moreover, ORP is another critical factor that helps assess the activation strength of PAW. High ORP values generally correlate with high levels of oxidative species like ozone ($O_3$) and hydrogen peroxide ($H_2O_2$). PAW's ORP ranges typycally from 400 to 900 mV or higher. A higher ORP indicates a more potent disinfectant or oxidizer, enhancing PAW's effectiveness [52]. There is a lack of consensus among various studies regarding the presence of peroxynitrites, specifically nitroxyl (HNO), its dimer hyponitrous acid ($H_2N_2O_2$), and peroxynitrous acid (ONOOH). Some researchers argue that these compounds are generated in post-plasma treatment, particularly through the oxidation of $NO_2^-$ by $H_2O_2$, and that they play a crucial role in the properties of PAW [39, 53–57]. Conversely, other publications suggest that these compounds have a maximum lifetime of only a few seconds after plasma shutdown, they do not consider them at all, or fail to detect them in the resulting PAW [37, 58, 59].

The purpose of this text is not to ascertain which studies are accurate or inaccurate. Furthermore, it is important to remind that the particles produced are highly dependent on the specific conditions under which the plasma is generated and the quality of the water used (distilled, rain or tap water). However, based on the aforementioned composition, it can be concluded that PAW exhibits disinfecting effects, primarily due to the presence of $H_2O_2$ and the reduction of pH resulting from $NO_2^-$ and $NO_3^-$. Additionally, as will be discussed in the following text, there are other biological effects on plants beyond these disinfectant properties.

## Effect of PAW on Plants

If dosed properly, PAW has demonstrated multiple beneficial effects on plants, however if dosed inappropriately, these effects can be the adverse ones. Firstly the beneficial ones are adressed. These effects can be classified into two main categories: nutritional effects, where PAW acts as a fertilizer, and biochemical effects, where it functions via signaling molecules [60]. The key reactive species, particularly nitrate ($NO_3^{-1}$) and hydrogen peroxide ($H_2O_2$), play crucial roles in enhancing plant growth and development, improving plant metabolism, and increasing stress resistance [60]. However, precise concentration control is essential, as excessive levels of these reactive species can induce severe abiotic stress and negatively impact seed viability, potentially harming plant development [23, 61]. Therefore, careful optimization of PAW treatment parameters is required for each specific crop type to maximize benefits while avoiding potential adverse effects.

Nitrogen is a fundamental biogenic element in photosynthetic plants, essential for amino acid (protein) synthesis and the conversion of inorganic substances into organic compounds. Plants primarily acquire nitrogen from soil solutions, with ammonia ($NH_3$) being a major source, typically supplied as fertilizers such as ammonium nitrate ($NH_4NO_3$) [62, 63]. Additionally, $NO_2^-$ and $NO_3^-$ serve as alternative nitrogen sources for green plants [62, 64, 65]. The presence of these components in PAW makes it a promising alternative fertilizer.

Nitrate assimilation can occur in any plant organ, with the energy for sequential nitrate reduction primarily derived from either photosynthesis in aerial organs or carbohydrate oxidation [66]. This reduction follows the pathway: $HNO_3 \rightarrow HNO_2 \rightarrow NH_2OH \rightarrow NH_3$.

The $H_2O_2$, another key component of PAW, is naturally produced in various plant cell compartments, including mitochondria, chloroplasts, cytoplasm, cytoplasmic membrane, peroxisomes, and cell walls [67, 68]. It is also generated during photosynthesis and respiration. It serves multiple functions in plants: it strengthens cell walls, regulates physiological processes (including senescence, photorespiration, photosynthesis, and cell cycle), and participates in both structural and biochemical defense mechanisms [68–70]. While $H_2O_2$ acts as a beneficial signaling molecule at low concentrations, high concentrations can trigger oxidative stress and potential cell death [68, 70].

As mentioned, some effects of PAW may be harmful to plants—typically due to nitrate overdose, nitrite toxicity, and affecting soil pH. Overdosing plants with nitrates ($NO_3^-$) has several negative effects on their growth and health [62–64]. Excessive intake of nitrates causes excessive vegetative growth, when plants produce a large amount of leaf mass at the expense of generative organs (few flowers, irregular flowering, lower fruit quality). The leaves are dark green, large, but fragile and susceptible to damage. Water management and mineral metabolism are disrupted. The entire plant may have a weakened immune system and be more susceptible to diseases and pests.

Nitrites ($NO_2^-$) pose a significant risk to plants because they are more toxic than nitrates and cause damage even at lower concentrations [62–64]. In general, nitrites disrupt metabolic processes, namely photosynthesis, respiration and energy metabolism. Plants have damaged chlorophyll and nutrient transport, which is why they are noticeable by yellowing of leaves, slowing down growth, dying off of roots and degradation of tissues. Toxicity caused by nitrites can lead to plant death, especially younger plants are very sensitive.

Finally, the use of PAW in agriculture is possible for seed treatment, as a foliar spray (fertilizer, against pests) or for soil irrigation [23, 44, 45, 71, 72]. The impact of the first two treatments is evident from the previous text. Long-term use of PAW for regular soil irrigation can disrupt the natural microbial balance in the soil, which can negatively affect its fertility. Excessive amounts of reactive oxygen species and nitrogen can also lead to damage to plants and soil organisms, can affect the pH of the soil, and can increase its acidity [44, 45, 70, 72]. Soils with a high buffering capacity can better withstand these pH changes, while soils with a low buffering capacity can be more susceptible to significant pH changes.

## Artificial PAW (APAW)

Let us now proceed to the primary objective of this text: to critically evaluate the optimistic conclusions presented in various papers. As mentioned at the outset, the central question is whether PAW can be artificially synthesized. If its composition is well understood, artificial preparation should be feasible. However, if the same effects are not replicated, further uncertainties regarding its composition may emerge. Currently, there is limited literature comparing PAW and APAW. The publications can be categorized into two groups: those in which APAW was not prepared as an exact imitation of PAW, and those in which the composition was closely matched.

In a paper by Wang et al. [73], the inactivation efficacy of PAW against *E. coli* and *Listeria innocua* was evaluated. $NO_3^-$ and $NO_2^-$ were detected in the original PAW; however, $H_2O_2$ was absent. The APAW solution was prepared using either hydrochloric acid (HCl) and nitric acid ($HNO_3$). $HNO_3$ was chosen to replicate the composition of PAW, while HCl served as a comparison with acidic media. Although APAW achieved a 2 $\log_{10}$ reduction in *E. coli*, its inactivation efficiency did not reach that of the corresponding PAW, which achieved up to a 5 $\log_{10}$ reduction. Chanioti et al. [74] prepared artificial PAW by dissolving $H_2O_2$, sodium nitrite ($NaNO_2$) as a source of $NO_2^-$, and potassium nitrate ($KNO_3$) as a source of $NO_3^-$ in deionized water at appropriate concentrations. However, the pH was not adjusted accordingly. The results indicated that the reduction of microorganisms, including *Brochothrix thermosphacta*, *Pseudomonas* spp., *Enterobacteriaceae*, hydrogen sulfide ($H_2S$)-producing microorganisms, yeasts/molds, and total aerobic bacteria in APAW-treated carp fillets, was only approximately 50–80% of that achieved with PAW treatment. Lee et al. [75] prepared APAW by dissolving sodium nitrite ($NaNO_2$), sodium nitrate ($NaNO_3$), and $H_2O_2$ in distilled water to achieve the desired concentrations. The pH was adjusted with sodium nitrate $NaNO_3$. The inactivation efficiency for bacteria such as *Bacillus cereus*, *Salmonella* spp., and *E. coli* on the surface of cherry tomatoes was approximately 1.3 $\log_{10}$ higher with PAW compared to APAW.

Because APAW in the three previous studies did not utilize identical concentrations of chemicals as the original PAW, the results cannot be deemed conclusive. However, other studies have successfully prepared APAW with concentrations that match those of PAW.

Zhou [76] detected $NO_2^-$, $NO_3^-$, $H_2O_2$, nitric oxide (NO), and ozone ($O_3$). After preparing APAW with the appropriate concentrations of all substances, the reduction in *E. coli* biofilm biomass was only approximately 45% for APAW, compared to approximately 60% for PAW. Kučerová et al. [60] irrigated lettuce plants using both PAW and chemically equivalent APAW, in which $H_2O_2$ and $NO_3^-$ were detected. After 5 weeks, the dry weight of the plants was very similar for both treatments and was approximately 15% higher compared to the control group. Jirešová et al. [71] investigated the inactivation of *E. coli* and the yeast *Saccharomyces cerevisae* on wheat grains, as well as the effects on germination, shoot length, and both fresh and dry shoot weight. In their study, the original PAW contained $NO_3^-$ and $H_2O_2$, while APAW was prepared as a mixture of $HNO_3$ and $H_2O_2$. The results of the experiments with PAW and APAW were comparable, with both achieving approximately a 2 $\log_{10}$ reduction in microbial counts. The study found minimal differences in the efficacy of PAW and APAW, confirming that there is no significant difference between the effects of the two treatments.

In conclusion, the APAWs prepared in the first group of studies, where pH was not controlled or where salts were utilized instead of $HNO_3$ and $HNO_2$, did not achieve the same efficiency as the original PAWs. Conversely, in the studies conducted by [60] and [71], where APAW was prepared using the appropriate compounds, no statistically significant differences were observed between PAW and APAW at the corresponding concentrations. However, Zhou [76] raises questions regarding the actual composition of PAW, as the reported lower efficacy of APAW compared to PAW remains significant and contradictory. This would suggest, that despite PAW and APAW are chemically similar, other chemical species which are not measured are generated during the plasma generation in the PAW.

## Economic Issue of the PAW and APAW Generation

The question of the identity of PAW and APAW can be set aside for now to focus on the economic aspects of potentially industrially generating PAW or its components, such as $HNO_2$, $HNO_3$, and $H_2O_2$, through electric discharges. Two components, $HNO_3$ and $H_2O_2$, can be purchased directly, with approximate prices of 8 euros per liter for 65% nitric acid and 7 euros per liter for 35% hydrogen peroxide. Nitrous acid ($HNO_2$) is not stable for extended periods and must be prepared shortly before use. For example, it can be produced by the decomposition of nitrites using strong acids, such us hydrochloric acid: $NaNO_2 + HCl \rightarrow HNO_2 + NaCl$. The approximate prices are 12 euros per kilogram for $NaNO_{2,}$ and 5 euros per liter for 35% HCl. For simplicity, ozone will not be considered in this discussion, as it is nearly impossible to store, and substantial research has not detected it in PAW.

For the PAW generation, electric discharge methods will be considered, considering only the cost of electricity, which is approximately 0.20 euro per kWh. In the study by Zhou [76], 200 mL PAW was generated using underwater microbubble discharge at 40 W for 15 min. This process resulted in an energy consumption of $E = 360$ J per 200 mL. After conversion, the resulting cost was 0.01 euros per liter of PAW. The cost of the chemicals required (excluding ozone) was approximately 0.0018 euros per liter. In the study of Jirešová [71], 1 mL of PAW was generated using corona discharge in transient spark mode, with a power of 2.7 W for 30 min. This corresponds to an energy consumption of $E = 4860$ J $= 0.00135$ kWh, resulting in a cost of 0.00027 euro per mL, or 0.27 euro per liter. The cost of the chemicals required was approximately 0.007 euro per liter. In the study by Kučerová [60], a transient spark corona discharge of 6 W was used, with 1 mL of PAW exposed for 1 min. The energy cost was 0.02 euro per liter, and the cost of chemicals was 0.0012 euro per liter.

A more detailed summary is presented in Table 1, which also includes the ratio of PAW production costs for discharge assistance to chemical costs. This ratio is approximately 5 for [76], 15 for [60] and up to 40 for [71].

A similar analysis was conducted from the perspective of energy efficiency, where the energy costs in the industrial processes for $HNO_3$ and $H_2O_2$ were chosen as 600 kJ/mol [77] and 1500 kJ/mol [78], respectively. Due to not direct production of $HNO_2$ and its negligible quantity, only the standard enthalpy of formation of 77 kJ/mol [79] was selected. The results are summarized in Table 2, which also indicates the inconvenient ratio of energy consumption ($E_{PAW}/E_{APAW}$) as 55, 108, and 174 for the same referenced works, consistent with the previous case.

The analysis indicates that producing PAWs or its components through electric discharges is economically inefficient. Consequently, industrial-scale production of these compounds via discharges is not cost-effective in its current form [77]. However, other factors may be considered. One significant advantage of discharge generation is the ability to

**Table 1** Economic inefficiency in the production of PAW and APAW

| | | Price (euro/L (kg)) | Zhou [76] | | Kučerová [60] | | Jirešová [71] | |
|---|---|---|---|---|---|---|---|---|
| | | | Used (mL, g) | Price (euro) | Used (mL, g) | Price (euro) | Used (mL, g) | Price (euro) |
| Chemicals APAW | $HNO_3$ 65% | 8 | 0.1000 | 0.000800 | 0.0602 | 0.0004816 | 0.602 | 0.004816 |
| | $H_2O_2$ 35% | 7 | 0.1100 | 0.000770 | 0.0400 | 0.0002800 | 0.323 | 0.002261 |
| | HCl 35% | 5 | 0.0200 | 0.000100 | 0.0300 | 0.0001500 | 0 | 0 |
| | $NaNO_2$ | 12 | 0.0138 | 0.000166 | 0.0260 | 0.0003120 | 0 | 0 |
| Sum | | | | 0.001836 | | 0.0012240 | | 0.007080 |
| Generation PAW | Power (W) | | | 40 | | 6 | | 2.7 |
| | Time (min) | | | 15 | | 1 | | 30 |
| | Volume (mL) | | | 200 | | 1 | | 1 |
| | Energy per liter (kJ/L) | | | 180 | | 360 | | 4860 |
| | Price per liter (euro/L) | | | 0.01 | | 0.02 | | 0.27 |
| Energy price 0.2 euro/kWh | | | | | | | | |
| Ratio electric/chemical price | | | 5.5 | | 16.3 | | 38.2 | |

**Table 2** Energetic inefficiency in the production of PAW and APAW

| | | Energy (kJ/mol) | Zhou [76] | | Kučerová [60] | | Jirešová [71] | |
|---|---|---|---|---|---|---|---|---|
| | | | Amount of substance per liter (mmol/L) | Energy per liter (kJ/L) | Amount of substance per liter (mmol/L) | Energy per liter (kJ/L) | Amount of substance per liter (mmol/L) | Energy per liter (kJ/L) |
| Chemicals APAW | $HNO_3$ | 600 [77] | 1.29 | 0.772 | 1.19 | 0.715 | 1.19 | 0.715 |
| | $H_2O_2$ | 1500 [78] | 1.66 | 2.484 | 1.72 | 2.581 | 13.89 | 20.84 |
| | $HNO_2$ | 77 [79] | 0.20 | 0.015 | 0.38 | 0.029 | 0.00 | 0.00 |
| Sum | | | | 3.271 | | 3.325 | | 27.987 |
| PAW | Energy per liter (kJ/L) | | | 180 | | 360 | | 4860 |
| Ratio $E_{PAW}/E_{APAW}$ | | | 55 | | 108 | | 174 | |

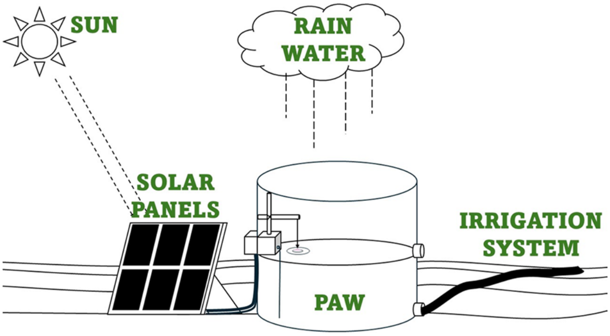

**Fig. 2** Illustration of the potential application of PAW in agriculture

produce PAWs on-site, which can be crucial in certain scenarios. Although electricity is necessary for this process, it can also be generated on-site. For instance, solar panels can be utilized, and PAW can be produced from rainwater (Fig. 2). The ecological scale can be a big plus, as the decentralized generation of PAW can result in a reduced transport [80].

In remote and inaccessible locations, generating PAW through discharge may be economically viable. Therefore, in certain specific cases, this approach might be justified. Another intriguing perspective on PAW involves storing energy in a chemical form, particularly when such energy is available at a low cost. Although direct transfer of energy back is not possible, it can still be utilized, for example, as a fertilizer, as previously discussed. Furthermore, PAW has potential applications for the wider general public where it is not appropriate to work with concentrated solutions of substances, because PAW could have potential as a on-site disinfectant, and may be prepared in small quantities only if that is sufficient. Another great potential is for example in medicine for treating skin wounds, e.g. in mice [34], and for the selective treatment of cancer cells, e.g. [35], as already mentioned. However, further exploration of these possibilities is beyond the scope of this paper.

## Conclusion

All the previous statements may sound optimistic and promising, but is the reality as miraculous as it appears? An analysis of PAW indicates that it primarily consists of $H_2O_2$ and $HNO_3$. This raises the question of whether PAW can be effectively substituted with an artificial alternative. Currently, there is a lack of publications comparing PAW with artificial substitutes, and the results from existing research are contradictory. If an artificial replacement is feasible, the significance of the PAW phenomenon may diminish. Conversely, if it cannot be replaced, the question arises regarding the presence of other particles in PAW, which remains an intriguing topic for further investigation.

This critical reflection concludes with a call to the scientific community to not only continue investigating PAW but also to compare experimental results with those obtained from APAW. Additionally, it would be advantageous to conduct further analyses of various PAW and to establish a standardized methodology for determining their composition and comparing it with that of APAW.

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## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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