

Rainwater-derived reactive oxygen species and their role in reducing environmental risks from arsenic in paddy rice systems

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The presence of various components in rainwater, and particularly of reactive oxygen species such as hydrogen peroxide (H_2O_2) and hydroxyl radicals ($\cdot\text{OH}$), has gained significant attention due to their impact on numerous biogeochemical functions and processes. Fan et al.^[1] recently published an article entitled 'Rainwater-Derived Reactive Oxygen Species Diminish Environmental Risk from Arsenic in Paddy Rice Systems'. The results from a mesocosm experiment showed that rainwater-borne hydrogen peroxide triggered hydroxyl radical ($\cdot\text{OH}$) generation, increased soil redox potential, and oxidized arsenite to the less phytoavailable arsenate in soil porewater, thereby reducing As uptake by rice and As accumulation in rice grains. On top of this very interesting finding, two key research issues remain unclear, i.e., the dissolution of various As-associated minerals, which is related to rain-induced high occurrences of H_2O_2 and $\cdot\text{OH}$, as well as the corresponding generation of less phytoavailable arsenate. We propose that oxygenic and anoxygenic photosynthetic activities by soil microorganisms might have played an important role in this framework. Microbial activities could be triggered by the uptake of various rainwater components, including nutrients, with a potential to generate significant amounts of H_2O_2 and $\cdot\text{OH}$ and to produce little phytoavailable arsenate in soil porewater.

Most importantly, Fan et al. (2025)^[1] conducted a mesocosm experiment to examine the effects of rainwater-borne H_2O_2 on As in soil porewater and rice grains. The experiment was carried out across two rice cropping cycles, at the experimental farm of the South China Agricultural University in Guangzhou, China. The primary objective of their study was to

confirm the well-established hypothesis that a Fenton-like reaction, triggered by the interaction between rainwater-borne H_2O_2 and iron (Fe) present in paddy soil, causes a decrease in As uptake by rice plants and grains. Additionally, the study investigated the impact of seasonal rainfall variation on As accumulation in rice grains. It also assessed the potential influence of spatial variation in rainfall regimes on the geographical distribution of As in rice grains across China's rice-producing regions. To achieve these objectives, the study simultaneously measured the concentrations of As, H_2O_2 , and hydroxyl radicals ($\cdot\text{OH}$), as well as microbial DNA in rainwater and the corresponding paddy soils. The soil redox potential (Eh) was also measured. The mesocosm experiment included two key treatments and one control. In the first treatment, designated T-rain, the growth cubicles were directly exposed to atmospheric precipitation. In the second treatment, designated T- H_2O_2 , the growth cubicles were irrigated with synthetic rainwater containing the same levels of H_2O_2 as the natural rainwater. The final treatment, serving as the control, involved irrigating the growth cubicles with the same amount of H_2O_2 -free irrigation water. Notably, the overlying water (OW) that was consistently maintained at approximately 2 cm depth and the soil-water interface were regularly sampled for subsequent measurements. The study showed that the H_2O_2 levels were substantially decreased, in both OW (H_2O_2 concentrations of ~ 1.5 to $2.2 \mu\text{M}$) and the soil-water interface (~ 0.75 to $2.0 \mu\text{M}$), in both the T-rain and the T- H_2O_2 treated soils, when compared to rainwater ($43.15 \mu\text{M}$ to $65.2 \mu\text{M}$) during the three different growth stages^[1]. In contrast, $\cdot\text{OH}$ radicals were significantly produced in both OW (~ 13.5 to $18.0 \mu\text{M}$ as cumulative concentration of generated $\cdot\text{OH}$) and the soil-water interface (~ 12.5 to $15.0 \mu\text{M}$) in both T-rain and T- H_2O_2 treated soils. In both cases, the levels of H_2O_2 and $\cdot\text{OH}$ radicals were significantly higher compared to those in control samples. These results clearly suggest the production of $\cdot\text{OH}$ radicals from H_2O_2 through the Fenton reaction^[1]. In treated samples, the average levels in rice

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grains of various arsenic species -including dimethylarsenate, monomethylarsenate, arsenite (As(III)), and arsenate (As(V))-were significantly lower in the T-rain and T-H₂O₂ samples compared to the control samples^[1]. Interestingly, As(III) was significantly decreased, while As(V) was increased in these treatments compared to the control samples. This result suggests that the decrease of arsenic in rice grains may result from the removal of labile arsenic species from soils, induced by ·OH. This redox process is further supported by the significantly higher abundances of As(III)-oxidizing bacteria and significantly lower levels of As(V)-reducing bacteria, along with significantly higher Eh levels, in the T-rain and T-H₂O₂ samples as compared to the control samples. Remarkably, a significant correlation between annual precipitation and grain-borne As across China's rice-producing regions ($p < 0.05$) provides strong evidence in favor of arsenic removal from rice grains in paddy soils on a national scale. The findings may offer valuable insights for assessing and managing the environmental risks associated with arsenic-contaminated rice grains. However, the described study did not explore the reasons behind the varying levels of H₂O₂ and ·OH radicals across different treatments, the dissolution of arsenic-associated minerals linked to rain-induced increases in H₂O₂ and ·OH, the resulting formation of less phytoavailable arsenate, or the occurrence of the photo-Fenton reaction during daylight hours in the OW.

An interesting aspect is represented by the fact that the observed levels of H₂O₂ decreased substantially from rainwater to the OW and, finally, the soil-water interface, possibly through H₂O₂ conversion into ·OH radicals by Fenton(-like) processes^[1]. The cumulated concentration of the produced ·OH radicals in the overlying water and at the soil-water interface (~4.3-15.5 and ~4.5-16.0 μM, respectively) during the first and second crop production indicates that a significant amount of H₂O₂ was missing in comparison with rainwater. In addition to Fenton and similar processes, enzymatic degradation of H₂O₂ might also play a significant role^[2,3]. Such process does not contribute to the generation of any ·OH radicals, but it produces water and O₂ (H₂O₂ + enzymes → H₂O + O₂). Therefore, a comparison between occurring H₂O₂ and generated ·OH in both overlying water and the soil-water interface might provide a hint into the relative roles of inorganic (Fenton) vs. enzymatic degradation of H₂O₂.

Most importantly, arsenic primarily occurs in various minerals such as iron (oxyhydr)oxides, clay minerals, and feldspars^[4,5,6]. The negatively charged ions AsO₃³⁻ and AsO₄³⁻ can donate electrons to the d-orbitals of Fe, which is similar to the electron donation from humic substance (HS) functional groups (e.g., carboxylic: -COO⁻; phenolic: -O⁻) to Fe's d-orbitals^[7]. In essence, the dissolution and/or mineralization of various As-containing minerals in the presence of rain-induced H₂O₂ and ·OH, along with the corresponding generation of less phytoavailable arsenate, remain unclear. In practice, soil microorganisms undergo rapid oxygenic and anoxygenic photosynthetic activities through symbiotic and non-symbiotic pathways^[8,9,10], possibly facilitated by the uptake of numerous reactive components from rainwater^[11], atmosphere^[9,12,13], and/or degradation byproducts of soil organic matter^[14,12,15,16]. This phenomenon involves continuous sequestration-redox processes that generate H₂O₂ and ·OH radicals^[17,18], which are primarily responsible for the dissolution and mineralization of As-

associated minerals in soils^[10,19]. These processes simultaneously produce photosynthetically-derived extracellular polymeric substances or humic substances (HS) components, which would contribute to the neoformation of HS-bound As-associated organo-minerals, thereby stabilizing both C and As^[1,10,20]. This hypothesis is further supported by the higher levels of both H₂O₂ and ·OH at the soil-water interface, which offset their enzymatic degradation compared to the overlying water.

The stable isotopes of δ¹³C_{DIC} (-22.2 ‰ to 0.2 ‰) and δ²H_{H₂O} (-350 ‰ to 64.9 ‰) in rainwater are relatively lighter or more depleted compared to the corresponding ones of soil water (-31.4 ‰ to 17.6 ‰ and -124 ± 8 ‰ to 20.7 ‰, respectively; reviewed in^[11]). Similarly, the occurrence of labile nutrients in rainwater, such as δ¹⁵N_{NH₃/NH₄⁺} (-28.7 ‰ to 16.2 ‰), δ¹⁵N_{NO₃-/NO_x} (-13.5 ‰ to 13 ‰), δ¹⁸O_{NO₃-} (-2.0 ‰ to 102 ‰), δ³⁴S_{SO₄²⁻} (-14.2 ‰ to 20.9 ‰), and δ¹⁸O_{SO₄²⁻} (5.0 ‰ to 41.7 ‰) is either partially depleted or partially enriched compared to those in soil water (respectively, -46.1 ‰ to 10.2 ‰, -48.6 ‰ to 20.4 ‰, -10.9 ‰ to 27.77 ‰, -28.7 ‰ to 16.2 ‰, -25.0 ‰ to 35.0 ‰, and -14.5 ‰ to 24.0 ‰), depending on the soil types^[11]. However, the high availability and lability of these nutrients in rainwater would promote both oxygenic and anoxygenic photosynthesis under high moisture conditions, as reported elsewhere^[8,10,21,22]. Remarkably, the depleted or lighter values of δD_{H₂O} facilitate its kinetic incorporation into the molecular structure of cyanobacterial cells, where at least 2700 water molecules are involved in growth and metabolism^[23]. Therefore, the preferential uptake of lighter or depleted δ¹³C_{DIC} and δ²H_{H₂O} in raindrops by soil microorganisms, compared to the enriched values in soil components, is kinetically favorable when there is a high availability of other labile nutrients. This process does not only facilitate the rapid uptake of the lighter CO₂ and/or DIC forms, but it also promotes the uptake of other nutrients present in raindrops. Such preferential uptake has also been observed in rivers, lakes or reservoirs, seawaters, soils, and desert sands^[11].

Simultaneously, sulfide-dependent photosynthetic S sequestration occurs^[9] and is induced by, for instance, lichen-associated cyanobacterial-fungal symbioses through COS uptake^[24], via irreversible redox reactions (e.g., COS + H₂O → 2H₂S + CO₂ → CH₂O + 2S⁰ + H₂O)^[25,8,13,26,10]. Importantly, soils simultaneously uptake and produce COS^[27,28,9,26], CO₂ or dissolved inorganic carbon (DIC: dissolved CO₂, H₂CO₃, HCO₃⁻, and CO₃²⁻)^[16,15,14], N₂O^[12,29,30], and H₂S^[13,31]. These processes occur through the photochemical and microbial degradation of soil organic matter^[14,32,33,26,34,31,10], as well as redox reactions within soils^[35]. In essence, intense redox activities would arise from sulfur and carbon sequestration, following the uptake of COS and/or H₂S^[9,26,36,27], through sequestration (S²⁻ ⇌ S⁰) and reduction-oxidation processes (redox: S⁰ ⇌ SO₄²⁻ ⇌ S²⁻/S₂²⁻ ⇌ SO₄²⁻)^[17] carried out by lichen-associated cyanobacterial-fungal symbiotic^[4] and non-symbiotic photosynthetic pathways^[10,37]. Concurrently, the eight electrons released from the redox reactions that transform sulfides into sulfates (S²⁻ → S⁰ + 2e⁻ and S⁰ → SO₄²⁻ + 6e⁻)^[17] would potentially generate reactive oxygen species (H₂O₂ and ·OH), and trigger the export of soil components originated from the mineralization and dissolution of various As-bound minerals^[33,38]. These natural sequestration-redox processes are affected by elevated lev-

els of H_2O_2 and $\cdot\text{OH}$ at the soil-water interface as compared to the OW,^[1] and they would be primarily responsible for the conversion of labile arsenite (AsO_3^{3-}) into less phytoavailable arsenate (AsO_4^{3-}) in soil porewater. An overall conceptual model is presented in Figure 1, which illustrates the sequestration or uptake of CO_2 , COS , DIC , or N -containing nutrients from rainwater by soil microorganisms, as well as the subsequent release of extracellular polymeric substances (EPS) or HS components and the neoformation of As-bound organo-minerals^[10,18]. Remarkably, the Fenton-like reaction is generally considered to occur in the dark, preferably at night, primarily in the OW of paddy soils. In contrast, the photo-Fenton reaction is more likely to occur predominantly during the daytime in the OW^[39,40,41], which coincides with high photosynthetic activity^[42,43,44]. This issue is further supported by experimental observations showing a decrease in Fe(II) levels upon light exposure, along with an increase in chlorophyll *a* (indicative of planktonic communities) in the OW of paddy soils^[42,44]. These changes are primarily responsible for the corresponding degradation of DOM released from photosynthetically-derived planktonic communities via the photo-Fenton reaction^[45,46,39]. However, this possible photo-Fenton process remains unclear in the original paper^[1].

Most importantly, the sequestration-redox processes might simultaneously produce photosynthetically-derived EPS, which rapidly convert into HS components that are responsible for the neoformation, stabilization, or sequestration of As-bound minerals^[10,19,20]. High photosynthetic activity, triggered by rain, may produce EPS or HS in high amount, which

may favor the formation of As-bound organo-minerals and yield less phytoavailable arsenate in soil porewater. This concept is further supported by experimental observations of As sequestration by photosynthetically-derived soil organic carbon (SOC) or of HS-bound As mineral neoformation during periods characterized by high-moisture and low-temperature conditions (25°C). In contrast, no As sequestration occurs in the dark, even in the presence of microbes, during day-night high-temperature fluctuations ($10\text{--}42^\circ\text{C}$)^[10]. A possible explanation is that high temperatures may lead to an increased dissolution and mineralization of HS-bound As minerals, resulting in a significant loss of mineral-associated dissolved organic carbon (DOC: 35.2%). In contrast, relatively low and constant temperatures may result in reduced mineralization of As-bound minerals, with a lower mineral-associated DOC loss (15.0%) observed after a 150-day incubation period^[10]. The described processes are expected to significantly decrease the availability of arsenate in (e.g., paddy) soil porewater and, consequently, in rice grains as well.

Recently, increasing levels of plastics and their additives (e.g., organophosphate esters) have posed a significant concern for soil pollution^[47,48,49]. However, the potential interactions of plastics and related compounds with soil organic matter and the sequestration-redox processes in As-contaminated or other types of soils remain uncertain. This issue should be the focus of further research.

Finally, rapid photosynthetic S and C fixation by soil microorganisms under wet conditions^[10,21,22] would be primarily attributed to the presence of various reactive components

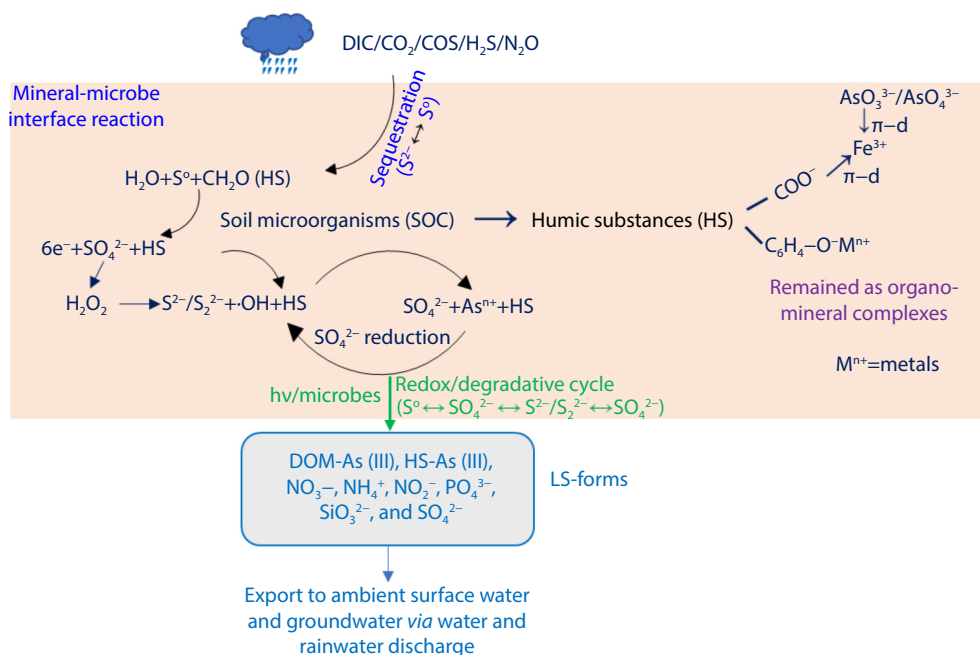


Fig. 1. A conceptual model for the sequestration or uptake of CO_2 , COS , H_2S , and/or N_2O during oxygenic and/or anoxygenic photosynthesis in the soil matrix. Photosynthetic uptake can occur either from the soil matrix or from the surrounding atmosphere, depending on the occurrence or absence of each gas in the relevant matrix. The uptake of COS or H_2S by soil microorganisms^[27,28,9] leads to simultaneous sequestration ($\text{S}^{2-} \rightleftharpoons \text{S}^0$) and reduction-oxidation processes ($\text{S}^0 \rightleftharpoons \text{SO}_4^{2-} \rightleftharpoons \text{S}^{2-}/\text{S}_2^{2-} \rightleftharpoons \text{SO}_4^{2-}$)^[27,51,17]. These processes yield photosynthetically-derived extracellular polymeric substances (EPS) or humic substances (HS) and contribute to the neoformation of As-bound organo-minerals in soils. The sequestration-redox process causes an export of labile-state (LS) forms of various components and causes mineral neoformation of the corresponding complexed-state (CS) forms. Notably, HS functionalities such as phenolates ($\text{C}_6\text{H}_5\text{--O}^-$) and carboxylates (COO^-) bind with metals (M, e.g., Fe^{3+}), for instance by donating electrons from their functional groups to the d-orbitals of Fe^{3+} , through a π -d electron bonding system. In complexed state (CS) organo-minerals, the negative electronic charges of arsenite (AsO_3^{3-}) and arsenate (AsO_4^{3-}) also bind with the d-orbitals of Fe^{3+} via the same π -d electron bonding system^[10,7].

in rainwater. This process is the primary pathway through which soil microorganisms preserve their energy, diversity, and biomass across various soil types and desert sands^[8,9,18,21,22,50,51]. In conclusion, a deeper mechanistic understanding of the production sources of H₂O₂ and ·OH, in relation to rain-induced generation of less phytoavailable arsenate, could further improve the findings of the original paper.

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Competing interests

The authors declare no competing financial interest.

Author contribution

K.M.G.M. designed and conceived the project; K.M.G.M wrote the manuscript; M.M., C.Q.L., N.S., G.S.S., and D.V. reviewed and edited the manuscript.

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