

Impacts of shale gas wastewater leaks on neighboring crops: Physiological and morphological responses of tomatoes

Original

Impacts of shale gas wastewater leaks on neighboring crops: Physiological and morphological responses of tomatoes / Li, Fengming; Yu, Xulin; Yang, Yushun; Tao, Wei; Zhu, Mengting; Zhang, Di; Shi, Shuling; Li, Huiqiang; Tang, Peng; Tiraferri, Alberto; Liu, Baicang. - In: PROCESS SAFETY AND ENVIRONMENTAL PROTECTION. - ISSN 0957-5820. - 195:(2025). [10.1016/j.psep.2025.106786]

Availability:

This version is available at: 11583/2998322 since: 2025-03-17T10:58:33Z

Publisher:

Elsevier

Published

DOI:10.1016/j.psep.2025.106786

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(Article begins on next page)

1 Impacts of shale gas wastewater leaks on
2 neighboring crops: physiological and
3 morphological responses of tomatoes

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16 **Abstract:** As shale gas extraction expands, the volume of flowback and produced water (FPW)
17 from hydraulic fracturing increases, raising concerns about the potential ecological risks of
18 leaks. In this work, we mixed tap water with different proportions of FPW to simulate various
19 leak scenarios. Tomatoes were irrigated using these mixtures or using FPW treated with multi-
20 stage pre-treatment and advanced membrane technologies to assess the effects on tomato seed
21 germination, plant vigor, element accumulation, and fruit gene expression. Compared to tap
22 water irrigation, all three dilution ratios of FPW inhibited seed germination and growth to
23 varying degrees, significantly reduced tomato biomass and fruit yield, and caused the fruit to
24 exceed safe limits for Pb and Cr. In the lowest dilution group, the germination rate was only
25 17%, and total biomass decreased by 55%. Even in the highest dilution group, fruit yield was
26 11% lower than the control. Additionally, the dilution showed high salt accumulation toxicity,
27 with yellowing at the edges of the leaves. Applying treated FPW accelerated tomato growth
28 and increased the yield of tomato hairs, with total biomass rising by 38%. Finally, a large
29 number of differentially expressed genes were detected in the fruits irrigated with FPW. These
30 genes are related to carbon and nitrogen metabolism, affecting the synthesis of carbohydrates
31 and proteins. These findings provide insights into the risks associated with shale gas FPW
32 leakage and offer guidance for the reuse of treated FPW.

33 **Keywords:** Shale gas wastewater, heavy metals, tomato growth, gene expression, food safety

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1 35 **1. Introduction**

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4 36 The combined use of conventional and clean energy is a key strategy for balancing energy
5
6 37 supply and environmental protection during the current energy transition. As natural gas prices
7
8
9 38 rise, interest in shale gas resources is growing. The level of hydraulic fracturing technology is
10
11
12 39 advancing, and industrial exploration and development of shale gas are rapidly expanding
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15 40 (Serrano-Areválo et al., 2022; Sun et al., 2019; Zhong et al., 2023). Upon hydraulic fracturing,
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17
18 41 fracturing fluids and formation water return to the surface in the form of flowback and produced
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21 42 water (FPW). Based on current annual FPW emissions in the Sichuan Basin, studies predict
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24 43 that by 2030, China will produce about 5 to 73 million cubic meters of FPW (Zou et al., 2018).
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27 44 This wastewater contains high concentrations of total dissolved solids, metals, organics,
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30 45 chemical additives, and naturally occurring radioactivity (Liang et al., 2022; Zhou et al., 2022).
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33 46 In recent years, there has been growing concern among communities and regulatory agencies
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36 47 about the potential impacts of FPW on agricultural activities, particularly on crop and soil
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39 48 quality, and it has become increasingly important to understand the interactions between FPW
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41
42 49 and agricultural systems (Ni et al., 2022; Peng et al., 2024; Zhou et al., 2023a).

43
44 50 Currently, FPW is treated by deep well reinjection, reused following some treatment, or
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47 51 discharged (Estrada and Bhamidimarri, 2016; Zhang et al., 2016). The deep well injection
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50 52 method involves treating FPW and reinjecting it into the same geological formation. However,
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53 53 this method faces issues such as limited injection well capacity, high uncertainty in injection
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56 54 risks, and increasingly strict water quality standards(Sun et al., 2019). Studies have shown that
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59 55 treating FPW through deep well injection can cause elevated concentrations of Cl, Br, Sr, and
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1 56 Li in nearby streams (Akob et al., 2016). The internal recycling method for treated FPW
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4 57 involves processing the wastewater to meet the quality standards for drilling or fracturing fluids,
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6 58 then reusing it in operations. This method offers benefits such as water conservation, low
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9 59 environmental impact, and low treatment costs. However, while repeated internal reuse has
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11
12 60 been shown to lead to enrichment of salinity and some pollutants in the recycled stream,
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15 61 causing pipeline corrosion and negatively affecting the production of gas wells (Zhou et al.,
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18 62 2022). After FPW is treated by physical or chemical methods to meet discharge standards,
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21 63 discharging it into surface water is the most widely used treatment approach. Current methods
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24 64 for treating shale gas wastewater include membrane separation, adsorption, advanced oxidation,
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27 65 activated sludge, and biofilm methods. Membrane-based technologies are considered a
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29
30 66 promising solution for treating such wastewater (Liu et al., 2024). Due to the complexity of
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33 67 shale gas wastewater composition, even after treatment, it may impact ecosystems in water
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35 68 used for crop irrigation and other purposes. (Song et al., 2024; Wu et al., 2023).

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37
38 69 Large volumes of FPW require consideration of the ecological risks of its leakage, which
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41 70 will inevitably result from storage pit and pipeline leaks, transportation accidents, or
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44 71 mishandling of surface discharges. The U.S. Environmental Protection Agency reported 457
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46
47 72 hydraulic fracturing-related spills across 11 states between January 2006 and April 2012, with
48
49
50 73 FPW spills being the most common type. Studies estimate that FPW transportation spills occur
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53 74 once every 19 well pads developed, and onsite spills occur once every 16 well pads (Clancy et
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56 75 al., 2018; EPA, 2015). Leaked FPW may enter streams through surface runoff and groundwater.
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59 76 Na and Cl are the two most concentrated substances in leaked FPW, and spills of saline
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1 77 wastewater can impact nearby streams for over six months. Studies monitoring FPW spills into
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4 78 rivers found high levels of Na downstream even six months after the incident (Agarwal et al.,
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6 79 2020). In January 2015, 11.4 million liters of natural gas wastewater spilled into a local river
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9 80 in the Williston Basin. Elevated concentrations of chlorides and bromides were detected
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12 81 downstream, with lithium, boron, and strontium levels 5–10 times higher than upstream.
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15 82 Radium activity in downstream sediments reached up to 15 times that of upstream levels
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18 83 (Cozzarelli et al., 2021; Cozzarelli et al., 2017). Even treated FPW poses risks to receiving
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21 84 water bodies. Organic pollutants persist in treated FPW and can be transported downstream
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23
24 85 (Zhou et al., 2024). Researchers found a significant increase in the relative abundance of 5
25
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27 86 antibiotic resistance gene (ARG) subtypes and 7 virulence factor genes (VFGs) at downstream
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30 87 sites near discharge points, indicating wastewater discharge impacts microbial communities
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33 88 (Mumford et al., 2020; Peng et al., 2024).

35 89 Plants can absorb and accumulate complex organic molecules, such as polycyclic
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38 90 aromatic hydrocarbons, endocrine disrupting chemicals, personal care products, and
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41 91 pharmaceuticals from soil and irrigation water (Dodgen et al., 2013; González García et al.,
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44 92 2019; Zhang et al., 2017). While FPW does not commonly contain any pharmaceuticals or
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47 93 personal care products, the salinity, alkalinity, and ionic composition of FPW may affect normal
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50 94 plant development and soil quality. Wheat plants have been shown to absorb and accumulate
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53 95 organic compounds from FPW, in turn affecting crop growth and causing wheat failure
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56 96 (Sedlacko et al., 2022; Shariq et al., 2021; Yang et al., 2024). FPW has also been shown to
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59 97 suppress plant immune responses and affect plant disease resistance (Miller et al., 2019).

1 98 Moreover, FPW may contribute to the accumulation of metals in soil and crops, thus affecting
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4 99 soil quality and posing risks to human health. A related study found that FPW caused significant
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6 100 accumulation of chloride and sulfate ions in soil, increased conductivity, and altered microbial
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9 101 communities, particularly near wastewater discharge sites (Zhou et al., 2023b). Another study
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12 102 analyzed 96 soil samples from shale gas extraction areas in China for toxic metals, revealing
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15 103 severe contamination with As, Cd, and Ni (Li et al., 2023). Overall, our understanding of the
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18 104 ability of plants to take up substances in FPW, the formation of toxic metabolites through plant
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21 105 metabolism, and the potential for these organic compounds to synergize with pesticides or
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24 106 inorganic compounds is limited (Cooper et al., 2022).

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26 107 In this study, tap water is mixed with different ratios of FPW to simulate different
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29 108 scenarios of potential leakage. The growth of tomatoes, fruit gene expression, and element
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32 109 accumulation levels are discussed upon irrigation with tap-FPW mixtures, treated FPW from
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35 110 wastewater treatment plants, condensate from the mechanical vapor recompression system, and
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38 111 tap water as control solution. This study aims to strengthen our understanding of the effects of
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41 112 FPW leakage on soil and crops, as well as to provide guidance for the safe reuse of treated
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43
44 113 FPW.

45 46 114 47 48 49 115 **2. Materials and methods**

50 116 **2.1. Water samples and water quality analytical methods**

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53 117 Seven types of aqueous solutions were used for irrigation, with the following
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56 118 denominations: 1) a “TW” tap water control; 2) “TFPW”: treated FPW from a shale gas
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1 119 wastewater treatment plant; 3) “MVR”: condensate from the mechanical vapor recompression
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4 120 system at the shale gas wastewater treatment plant; 4) “FPW”: undiluted flowback and
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6 121 produced wastewater; 5) “FPW5” (intense leakage scenario): FPW diluted in a ratio of 1:4 with
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9 122 TW; 6) “FPW10” (medium leakage scenario): FPW diluted in a ratio 1:9 with TW; 7) “FPW20”
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11
12 123 (weak leakage scenario): FPW diluted in a ratio of 1:19 with TW.
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15 124 The raw shale gas FPW used in this study was obtained from a shale gas well in Yibin,
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18 125 Sichuan, China. The TFPW and MVR were collected from a shale gas wastewater treatment
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20
21 126 plant in Yibing, Sichuan, China. The treatment plant comprises a multistage pretreatment
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23
24 127 combined with biotreatment and membrane-based separation (**Fig S1**) (Supporting
25
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27 128 Information), and the treated effluent meets the first-level standard for integrated wastewater
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29
30 129 discharge (GB8978-1996). The MVR system mainly consists of the following components:
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33 130 steam compressor, evaporative condenser, preheater, and various pumps for supplying power
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36 131 to the feed and discharge. The system operates at an evaporation temperature of 75.4°C, a
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39 132 condensation temperature of 85.5°C, and produces 12 m³ of condensate per hour. Description
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41
42 133 of the analytical methods for the determination of total dissolved solids (TDS), total nitrogen
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44 134 (TN), dissolved organic carbon (DOC), ions and elements can be found in **Text S1**.
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47 135 48 49 136 **2.2. Greenhouse experimental design**

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52 137 Tomato, which is widely grown in the Sichuan Basin, was chosen as the focus crop of this
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55 138 work. The tomato seeds and planting soil were purchased from Lanxiang Horticultural Seed
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58 139 Co., Ltd (Gansu, China).
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1 140 The greenhouse investigation was conducted in a laboratory of Sichuan University, Yibin
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4 141 Campus, using full-spectrum fill-in lamps for supplemental illumination, and with controlled
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6 142 ambient temperature values between 18°C and 25°C. First, a ten-day seed germination
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9 143 experiment was conducted in Petri dishes. Specifically, germination experiments were
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12 144 conducted in a greenhouse with 30 seeds per treatment group, equally divided into three Petri
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15 145 dishes, each with a qualitative filter paper and 8 mL of corresponding irrigation water, which
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18 146 was changed every 24 h. The number of seeds germinated in each group was recorded every
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21 147 48 h and the germination percentage was calculated. The length of the embryonic axis and root
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23
24 148 were measured using a centimeter ruler, and the fresh weight of the seeds was weighed on the
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27 149 tenth day using an electronic balance.

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29 150 After 10 days of cultivation in the Petri dishes, four tomato seedlings were selected from
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31
32 151 each group and transferred into rectangular seedling boxes for cultivation, with the length and
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34
35 152 width of each rectangle being 3 cm, and the depth being 5 cm. The seedlings were incubated
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37
38 153 in rectangular boxes for 30 days and irrigated every 7 days with 50 mL of solution. On the
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41 154 thirtieth day, the seedlings entered the four-leaf stage and were moved into 3-gallon plastic pots
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43
44 155 for stabilized culture, irrigated using 100 mL of solution every 72 h for 90 days in total. Each
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47 156 tomato plant used approximately 3.5 L of corresponding irrigation water during the whole
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50 157 cultivation period (**Fig S2 and Fig S3**).

51 52 158 53 54 55 159 **2.3. Morphological measurements and harvesting**

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58 160 The height of the seedlings above the soil surface was measured every 15 days after the
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1 161 seedlings were planted. On days 60 and 120, the leaf area of each tomato plant was measured.

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4 162 When the tomatoes were ripe, the plants were stripped from the soil and washed with ultrapure

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6 163 water, then dried in a freeze-dryer for 36 h. The above and below-ground biomass of each plant

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9 164 was determined. Soil samples were collected from the roots of mature tomato plants, along

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12 165 with a small amount of soil that was not used for the cultivation of the plants. The soil samples

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15 166 were crushed with a mortar and pestle, filtered through a 100-mesh nylon sieve, and finally

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18 167 dried with a freeze dryer for 36 h.

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22 23 24 169 **2.4. Soil and tomato tissue extraction and analysis via ICP-MS**

25
26 170 100 mg of dried samples were added to polytetrafluoroethylene (PTFE) digestion tubes,

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29 171 followed by 70% nitric acid (6mL) and hydrogen peroxide (1 mL), then the digestion was

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32 172 conducted using a microwave dissolver. The digestion temperature program was the following:

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35 173 ramp from room temperature to 120 °C in 5 min, hold for 1min, ramp to 160 °C at 8 °C/min

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38 174 rate, hold for 5 min, ramp from 160 °C to 180 °C at 5 °C/min rate, hold for 10 min. After

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41 175 digestion, samples were cooled, diluted to 50mL with ultrapure water, and finally filtered using

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44 176 a 0.22 µm PTFE filter into glass bottles that had been pre-washed with acid. The heavy metal

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47 177 concentrations were quantified with an inductively coupled plasma mass spectrometer

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50 178 (NexION 1000GPerkinElmer, Inc., MA, USA).

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54 55 180 **2.5. Gene expression of tomato fruit**

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58 181 Fresh fruits weighing slightly more than 1 g were taken from each irrigated group during

1 182 the fruiting stage. These fruits were then washed with DEPC water, packed into enzyme-free
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4 183 freezing tubes, immediately frozen in liquid nitrogen, and stored at -80°C until RNA
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6 184 extraction and transcriptome sequencing. Detailed information about RNA extraction,
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9 185 sequencing, read mapping, differential expression analysis, and functional enrichment were
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12 186 summarized in **Text S2**. Raw sequencing data were uploaded to the NCBI database under the
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14
15 187 biological project number PRJNA1197360.

18 188

21 189 **2.6. Statistical analysis.**

24 190 Data analysis was performed using Microsoft Excel 2010 and graphing was done using
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26 191 Origin 2022 (OriginLab, Hampden, MA). Statistical significance was analyzed through one-
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28
29 192 way analysis of variance (ANOVA, $p < 0.05$).

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35 194 **3. Results and discussion**

38 195 **3.1. Water quality analysis**

41 196 **Table 1** summarizes the general water quality of the seven irrigation solutions. Total
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44 197 dissolved solids (TDS) and various metals at substantial concentrations are found in FPW,
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47 198 which may have negative effects on plant health and disrupt the ecological balance of soil and
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50 199 water (Golding et al., 2022; Isayenkov and Maathuis, 2019; Nikalje and Suprasanna, 2018; Sun
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53 200 et al., 2021; Xie et al., 2022). The raw FPW used in the experiments, as well as FPW5, FPW10,
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56 201 and FPW20, did not comply with Chinese and FAO irrigation standards. Except for elemental
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58
59 202 Sr, the concentrations of heavy metals in FPW5, FPW10, and FPW20 were within the FAO

1 203 limits for heavy metals, but the salinity of all three types of aqueous mixtures was high.
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4 204 Specifically, the salinity of FPW20, the most diluted solution, was 1520 mg/L and exceeded
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6 205 the Chinese standard for irrigation water for agricultural fields, namely, 1000 mg/L. Note that
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9 206 even low concentrations of metals in irrigation solutions may lead to substantial accumulation
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12 207 in plants (Oetjen et al., 2018; Sedlacko et al., 2020). The water quality of TW, TFPW, and MVR
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14
15 208 met instead Chinese and FAO irrigation standards. TFPW had lower heavy metal
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17
18 209 concentrations than TW, except for Ba (0.35 mg/L) and Co (0.024 mg/L). The concentration of
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21 210 total nitrogen (71.4 mg/L) in MVR was significantly higher than that in TW (1.28 mg/L); note
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24 211 that a large amount of nitrogen could increase tomato fruit yield (Bénard et al., 2009; Trandel
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26
27 212 et al., 2018). The water quality analysis in this study was limited to two inorganic families of
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30 213 constituents, salinity and metals, while toxic organic matter in FPW can also pose a threat to
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32 214 crop safety and would require further study.
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215 **Table 1.** Comparison of water quality of 7 irrigation solutions (TW, TFPW, MVR, FPW, FPW5, FPW10, FPW20) with national and international
216 irrigation guidelines

Parameter	TW	TFPW	MVR	FPW	FPW5	FPW10	FPW20	water quality for irrigation (China)	water quality for irrigation (UN)
Turbidity (NTU)	0.17	0.65	1.62	198	54.9	10.0	7.58	/	/
DOC (mg/L)	1.52	2.37	6.67	181	34.4	16.9	8.39	/	/
TN (mg/L)	1.28	0.518	71.4	56.2	11.2	5.94	3.33	/	/
UV ₂₅₄ (cm ⁻¹)	0.011	0.006	0.012	1.42	0.132	0.323	0.596	/	/
TDS (mg/L)	251	392	272	24500	4890	2400	1520	1000	2000
pH	7.78	8.04	8.36	7.91	7.17	7.07	7.09	5.5~8.5	6.5~8.4
K (mg/L)	2.58	0.936	0.187	135	29.7	13.8	7.96	/	/
Ca (mg/L)	30.4	3.31	1.24	577	119	60.1	33.2	/	400
Na (mg/L)	2.14	9.70	1.10	6780	1380	695	351	/	920
Mg (mg/L)	27.0	2.51	0.687	104	22.4	10.1	4.07	/	60
Ba (mg/L)	0.222	0.351	0.054	33.6	7.12	3.86	1.83	/	/
Sr (mg/L)	0.137	0.075	0.046	59.3	22.7	10.6	4.98	/	0.2
Fe (µg/L)	438	37.8	/	1340	258	127	57.8	/	5000
Mn (µg/L)	6.47	1.26	2.45	506	103	53.4	29.2	300	200
Cu (µg/L)	0.288	0.283	/	77.4	15.9	8.70	5.48	500	200
Zn (µg/L)	14.7	3.42	1.79	9.45	10.3	11.5	13.1	2000	2000
Ni (µg/L)	4.96	2.01	0.264	8.48	7.14	6.26	5.93	100	200

Cr (µg/L)	0.790	0.012	0.064	21.8	3.62	1.64	0.834	100	100
Pb (µg/L)	0.020	0.020	/	2.67	0.593	0.314	0.175	200	5000
Cd (µg/L)	0.013	0.010	/	0.416	0.097	0.062	0.035	10	10
Co (µg/L)	0.003	0.024	/	0.310	0.057	0.023	0.012	1000	50
NH ₄ ⁺ (mg/L)	0.012	/	77.9	55.6	5.67	2.59	0.903	2	6
F ⁻ (mg/L)	0.129	0.075	/	9.88	2.34	0.981	0.398	2	1
Cl ⁻ (mg/L)	51.1	155	20.2	31800	6602	4190	2660	350	350
Br ⁻ (mg/L)	2.42	0.006	0.247	94.5	72.5	54.6	42.0	/	/
NO ₃ ⁻ (mg/L)	3.67	0.891	0.395	45.3	27.9	15.2	8.92	/	10

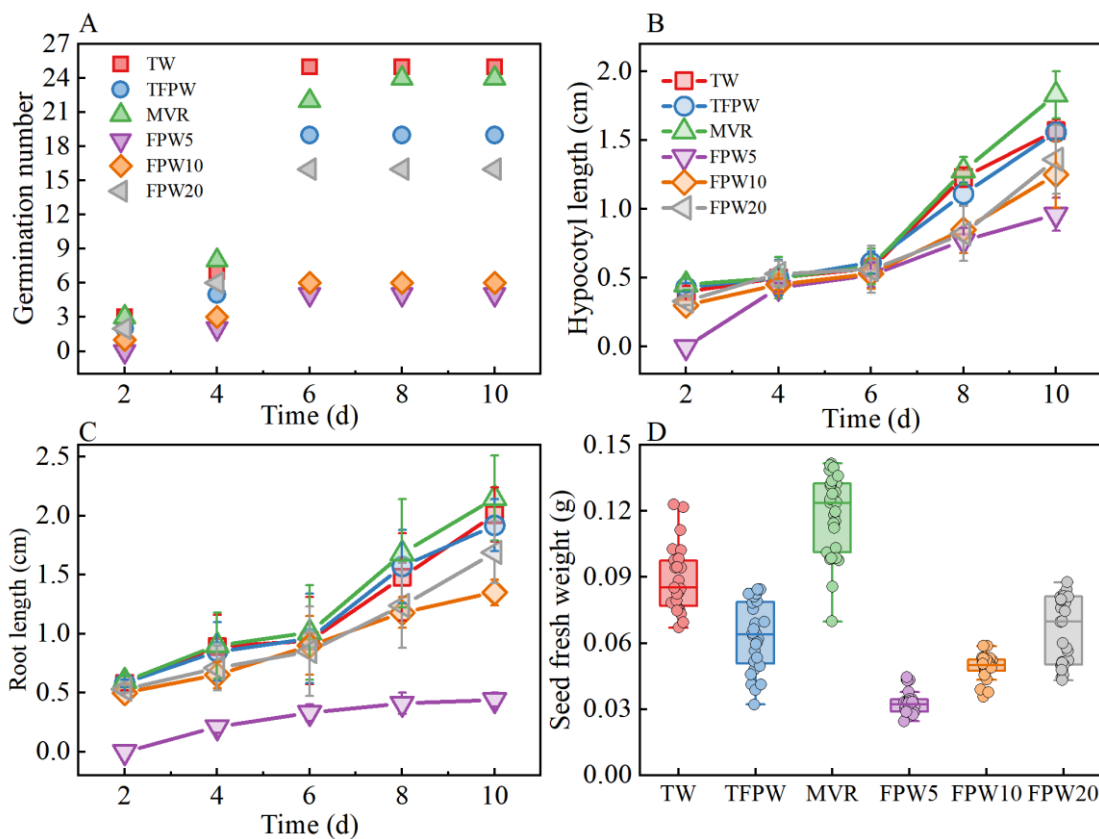
217 Note: NA: not available; TFPW: treated flowback and produced water; TW: tap water; FPW5: untreated FPW diluted 1:4 with TW; FPW10:
 218 untreated FPW diluted 1:9 with TW; FPW 20: untreated FPW diluted 1:19 with TW; DOC: dissolved organic carbon; TN: total nitrogen; TDS:
 219 total dissolved solids.

220 3.2. Effects on the germination and growth of tomato seeds

221 We conducted a 10-day experiment to evaluate the effect of different irrigation
222 solutions on tomato seed germination. The results are summarized in **Fig 1**, including
223 the germination number, hypocotyl length, root length, and seed fresh weight. At the
224 end of the ten-day germination experiment, the MVR group had a comparable
225 germination rate to TW group, both exceeding 80%, while the other solutions
226 containing raw FPW at different ratios produced lower germination numbers. In
227 particular, FPW5 and FPW10, the two mixed solutions containing the larger fractions
228 of raw FPW, consistently provided worse results, with germination rate as low as 17%
229 and 20%, respectively (**Fig 1A**). The experimental results were similar to previous
230 results of FPW used for plant irrigation (Chang et al., 2020; Novichkova et al., 2021).

231 The poor germination performance of seeds in the TFPW group may be related to
232 the higher levels of Na and Cl in TFPW compared to TW. These ions can affect the
233 seed's ability to absorb water and nutrients, thereby impacting seed physiological
234 functions. The low germination rate in the FPW groups can be attributed to their high
235 conductivity and salinity. During germination, salinity may cause irreversible toxic
236 effects. High conductivity and salinity disrupt the osmotic relationship between seeds
237 and water, reducing the amount of water absorbed for germination (Jacob et al., 2024).
238 On the other hand, Yang et al found that untreated FPW inhibits seed germination and
239 growth due to its high toxicity, which also limits nutrient absorption by the seeds. (Yang
240 et al., 2022). Compaoré et al showed that distilled water, which contains few minerals

241 and very little salt, accelerates the germination and growth of tomato seeds (Compaoré
 242 et al., 2024). MVR irrigation not only results in a germination rate similar to that of TW,
 243 but also promotes seedling hypocotyl length, root length, and weight. This is likely
 244 because MVR contains fewer minerals and small amounts of salt, which are more easily
 245 absorbed by the seeds. Although germination mainly depends on water quality,
 246 environmental factors and the inherent conditions of the seeds may also influence
 247 germination.



248
 249 **Fig 1.** Germination of tomato seeds in different irrigation groups. (A) Number of
 250 germination seeds; (B) Hypocotyl length; (C) Root length; (D) Seed fresh weight at the
 251 end of germination experiment.

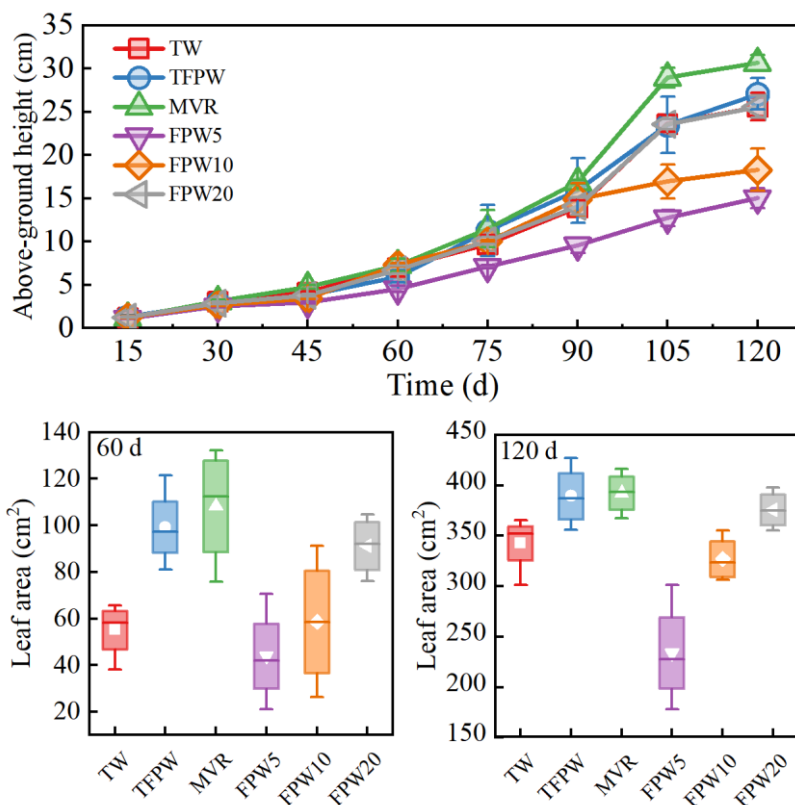
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1 253 **3.3. Effects on tomato growth and harvesting**

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4 254 Tomatoes were cultivated for 120 days in 3-gallon plastic pots before being
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6 255 harvested. Among the six irrigation groups, using MVR marginally increased the
7
8
9 256 average plant height and leaf area, while tomatoes irrigated with TFPW and FPW20
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11
12 257 showed no significant difference in plant height compared to the TW control group (**Fig**
13
14
15 258 **2**). Compared to TW, FPW5 and FPW10 irrigation significantly decreased plant
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18 259 biomass and fruit yield (Figure 3). Due to higher dilution, FPW20 had similar biomass
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21 260 levels to TW, but its fruit yield was 11% lower than the control. Irrigating with TFPW
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24 261 and MVR increased total biomass by 38% and 46%, respectively. Interestingly, the
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26
27 262 TFPW group performed much better in the pot experiment than in the petri dish
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30 263 germination test, suggesting that treated shale gas wastewater can be reused for
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33 264 irrigation without severely affecting plant health. The differences in seed germination
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36 265 and plant growth may be due to varying cultivation conditions, which aligns with
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39 266 previous reports on crop cultivation (Yang et al., 2022). Nitrogen is one of the most
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42 267 important nutrients for plant growth, and adequate nitrogen supply promotes leaf
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45 268 growth in tomatoes, increasing overall photosynthetic capacity(Batelli et al., 2024).
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48 269 MVR had a total nitrogen content of 71.4 mg/L, and the highest biomass in the MVR
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51 270 group may be due to the high nitrogen content in the water.

52
53 271 The reduction in tomato biomass and fruit yield in the three FPW treatment groups
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56 272 may result from the combined effects of high salinity and ion concentration in FPW. In
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59 273 FPW5, FPW10, and FPW20, the concentrations of most cations, except Sr and Na, were

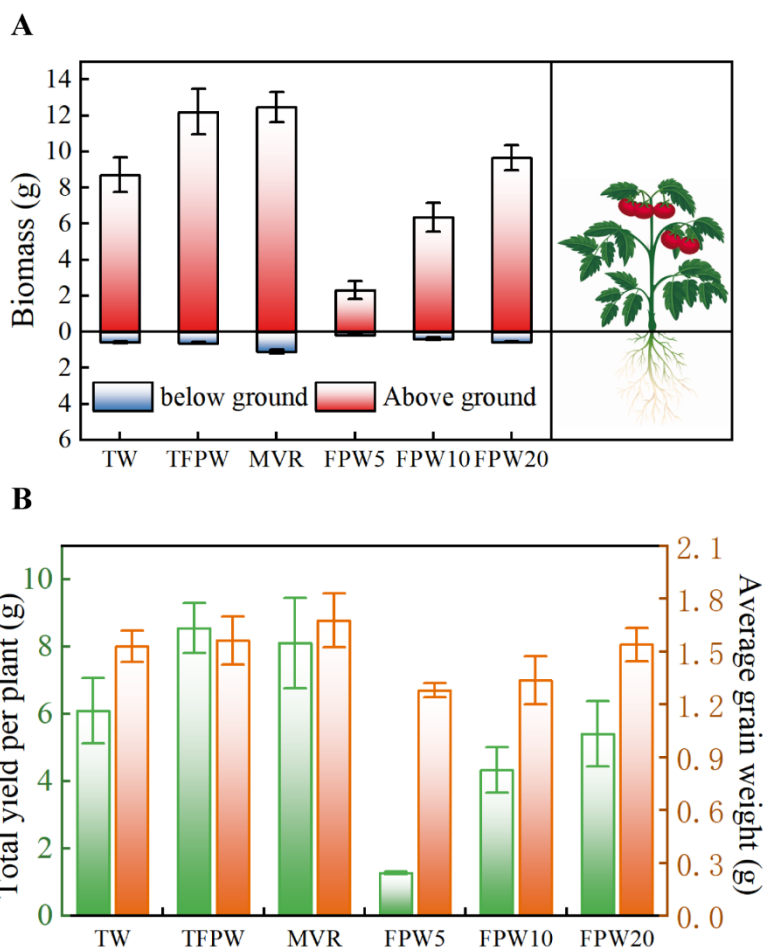
274 within irrigation standards. However, compared to TW, the concentrations of ions like
 275 K, Ca, and Na were generally higher. High ion concentrations can cause competition
 276 between different ions, which affects the plant's nutrient uptake (Rodrigues et al., 2016).
 277 Some ions, such as Cu and Mn, can be toxic at high concentrations and may inhibit
 278 physiological processes like photosynthesis and cell division. Under salt stress, the
 279 plant's ability to absorb nutrients through the roots decreases, which affects root
 280 growth (Ma et al., 2020). The leaves may suffer from burning, yellowing, and growth
 281 stagnation, leading to reduced biomass. FPW5 had a salinity of 4890 mg/L, and at such
 282 high salinity, clear developmental setbacks were observed, with a 55% reduction in
 283 tomato biomass. In FPW10, tomato leaves showed signs of burning (Fig S3).



285
 286 **Fig 2.** (A) Above-ground height of the tomato irrigated with different waters in the soil.

287 (B) Statistics of leaf area of tomato plants in different irrigation groups on days 60 and
 288 120. (After transferring from the Petri dishes into soil for cultivation, four plants from
 289 each group).

290



291

292 **Fig 3.** (A) Aboveground biomass (red) and belowground biomass (blue) of different
 293 irrigation groups after harvesting. (B) Total tomato fruit yield per plant (green) and
 294 average fruit weight (orange).

295

296 3.4. Effects on element accumulation in soil

297 As irrigation time increases, constituents in the irrigation water may accumulate

1 298 in the soil and alter soil quality. To investigate the possible accumulation of elements in
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4 299 soil from irrigation solutions, we measured the content of 12 elements (K, Ca, Mg, Cd,
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6 300 Sr, V, Zn, Cr, Ni, Pb, Cu, Co) in the original soil and in the soil after plant harvest. As
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9 301 shown in **Fig S4**, elemental concentrations increased to varying degrees in each
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12 302 irrigation group compared to the original soil, and the growth trend was related to the
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15 303 elemental concentrations in the irrigation water. No significant differences in soil heavy
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18 304 metal concentrations were observed with TW or MVR. Except for Zn and Mg, FPW5,
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21 305 FPW10, and FPW20 had higher elemental accumulation than TW. For example, V and
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24 306 Sr in soils irrigated with FPW5 were 41% and 18% higher than TW, respectively.
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27 307 Although irrigation with diluted FPW resulted in higher heavy metal soil content, none
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30 308 of the values exceeded the maximum permissible limits set by the Chinese Soil
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33 309 Environmental Quality Standards and FAO (Bakari et al., 2022; Yang et al., 2022).
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36 310 However, if FPW leaks over a long period, the concentration of heavy metals in the soil
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39 311 may gradually accumulate. This could negatively impact soil microbial structure and
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41
42 312 diversity. Additionally, these metals may be absorbed by plants or consumed by animals,
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45 313 leading to contamination of the soil, food chain, and groundwater, ultimately affecting
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48 314 public health. (Li et al., 2024; Ren et al., 2024).

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51 316 **3.5. Effects on element accumulation in tomato tissues.**

52 317 The results in terms of elemental content in tomato roots, stems, leaves, and fruits
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55 318 are shown in **Fig 4** and **Fig S5**. The trend of accumulation of different elements in
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1 319 different parts of the tomato varied, but irrespective of the type of irrigation, Ba, Co, Cr,
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4 320 Mn, Zn, and Ni accumulated more in tomato roots than in other parts of the tomato. All
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6 321 other elements accumulated in stems and leaves, and the transport of these elements to
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9 322 the fruits seemed limited.

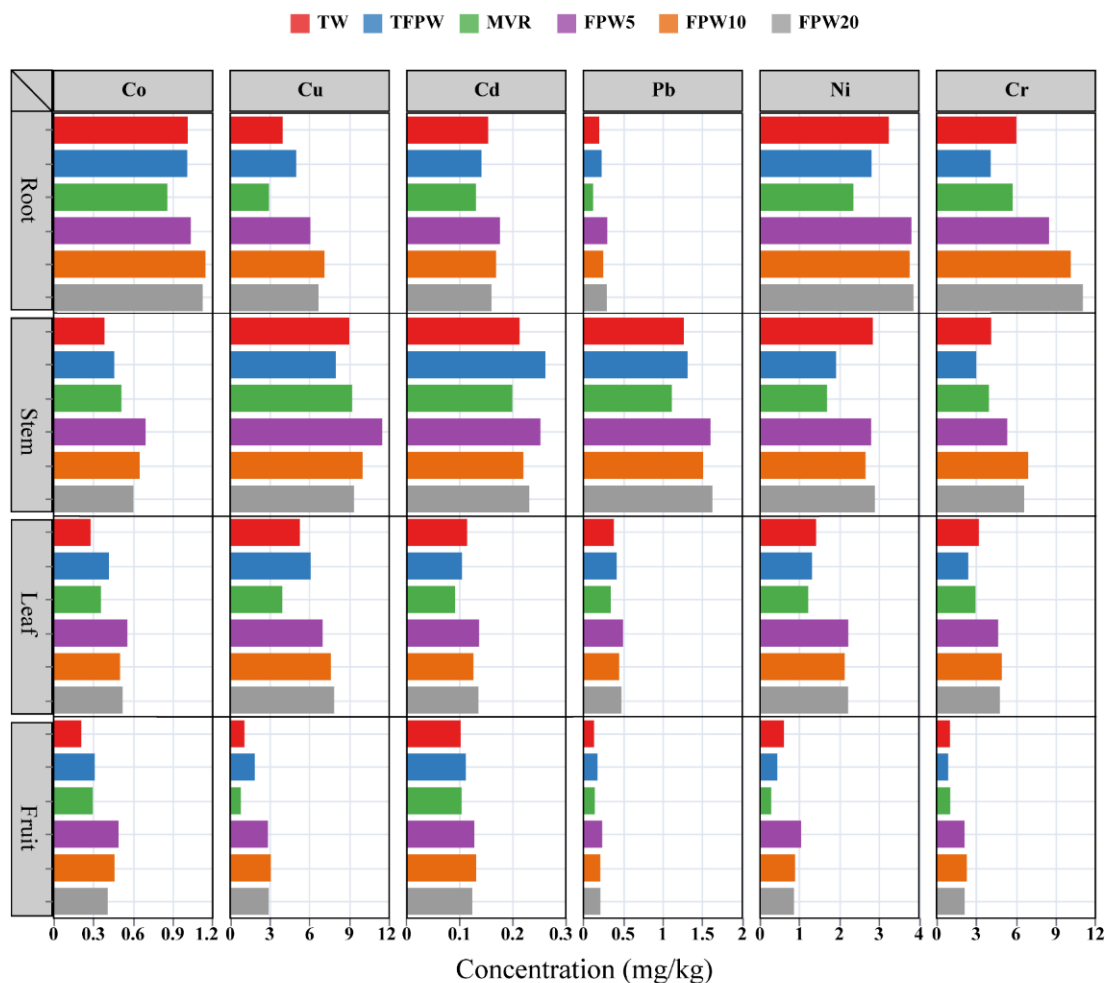
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11
12 323 Irrigation solutions containing FPW at different proportion were associated with
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15 324 the most important elemental accumulation in various parts of the plants. In detail,
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18 325 tomatoes irrigated with FPW5, FPW10, and FPW20 accumulated higher levels of Cd,
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21 326 Cu, Pb, Co, Cr, Mn, and Ni, with Cd, Cu, and Pb accumulating in large quantities in the
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24 327 stems and leaves, and the other elements in the tomato root system. Although FPW5
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27 328 had the highest levels of each substance, tomato tissues irrigated by FPW5 did not show
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30 329 the highest elemental concentrations among the six groups. These results align with
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33 330 previous findings and other studies suggesting that high aqueous concentrations may
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36 331 poison plants and affect the uptake and transport of elements (Kumari et al., 2015;
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39 332 Sedlacko et al., 2019; Yang et al., 2022). Interestingly, TFPW-irrigated plants contained
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42 333 lower elemental content than TW, except for Co, likely owing to the lower levels of
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45 334 elements in treated FPW, again suggesting the potentially safe reuse of this stream for
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48 335 irrigation. MVR-treated plants contained instead higher levels of Ca, Mg, and Zn
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51 336 compared to plants irrigated with TW. Specifically, the high ammonia-nitrogen content
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54 337 of MVR promoted tomato plants growth, resulting in more robust root systems and
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57 338 enhanced nutrient absorption.

58 339 The tomato root system plays a crucial role in the chelation and adsorption of
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1 340 heavy metals, with metal ions being transported through the root cell membranes into
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4 341 plant tissues. (Page and Feller, 2015). High salinity water generally increases the uptake
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6 342 of heavy metals in plants, and in phytoremediation, salinity is often used to enhance
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9 343 metal bioavailability, while salinity inhibits the uptake of nutrients (Munns and Tester,
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12 344 2008; Nikalje and Suprasanna, 2018; Sedlacko et al., 2020). However, in this work,
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15 345 tomatoes irrigated with FPW20 exhibited higher nutrient content than the control (TW).
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18 346 This may be explained by the fact that, under salinity stress, an imbalance in water
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21 347 concentration across the plant cell membranes disrupts ionic balance, and the plant may
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24 348 tend to increase the uptake through the root system to resist the osmotic pressure
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27 349 difference caused by the salinity stress, in which case the plant is forced to selectively
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30 350 absorb more nutrients (Acosta-Motos et al., 2017). Additionally, the transport of many
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33 351 heavy metals is regulated by the same transporter proteins, creating competitive
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36 352 relationships in metal accumulation: e.g, Cd, Ca, Mg, Cu, and Zn are absorbed through
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39 353 the same transporter proteins (Ghugre et al., 2023; Ismael et al., 2019; Zhou et al., 2016).
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42 354 The complex interrelationships between metals, nutrients, and salt need to be followed
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45 355 up with further, in-depth studies.

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47 356 Plants, as the first consumers in the food chain, absorb metal elements through
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50 357 their roots and accumulate them in their tissues. These metals can then transfer up the
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53 358 food chain, affecting other organisms that consume the plants, such as insects, small
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56 359 animals, and humans. Elevated levels of Cr and Pb, exceeding food safety standards,
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59 360 were detected in the tomato fruits from the three FPW-containing groups. Consuming

361 tomatoes exposed to FPW could cause serious acute and chronic health risks for humans.
 362 Cr may lead to chronic health issues, such as affecting glucose metabolism and
 363 cardiovascular health. Pb toxicity increases with each generation, and even low-level,
 364 long-term exposure can accumulate and cause health problems(Chowdhury et al., 2024).

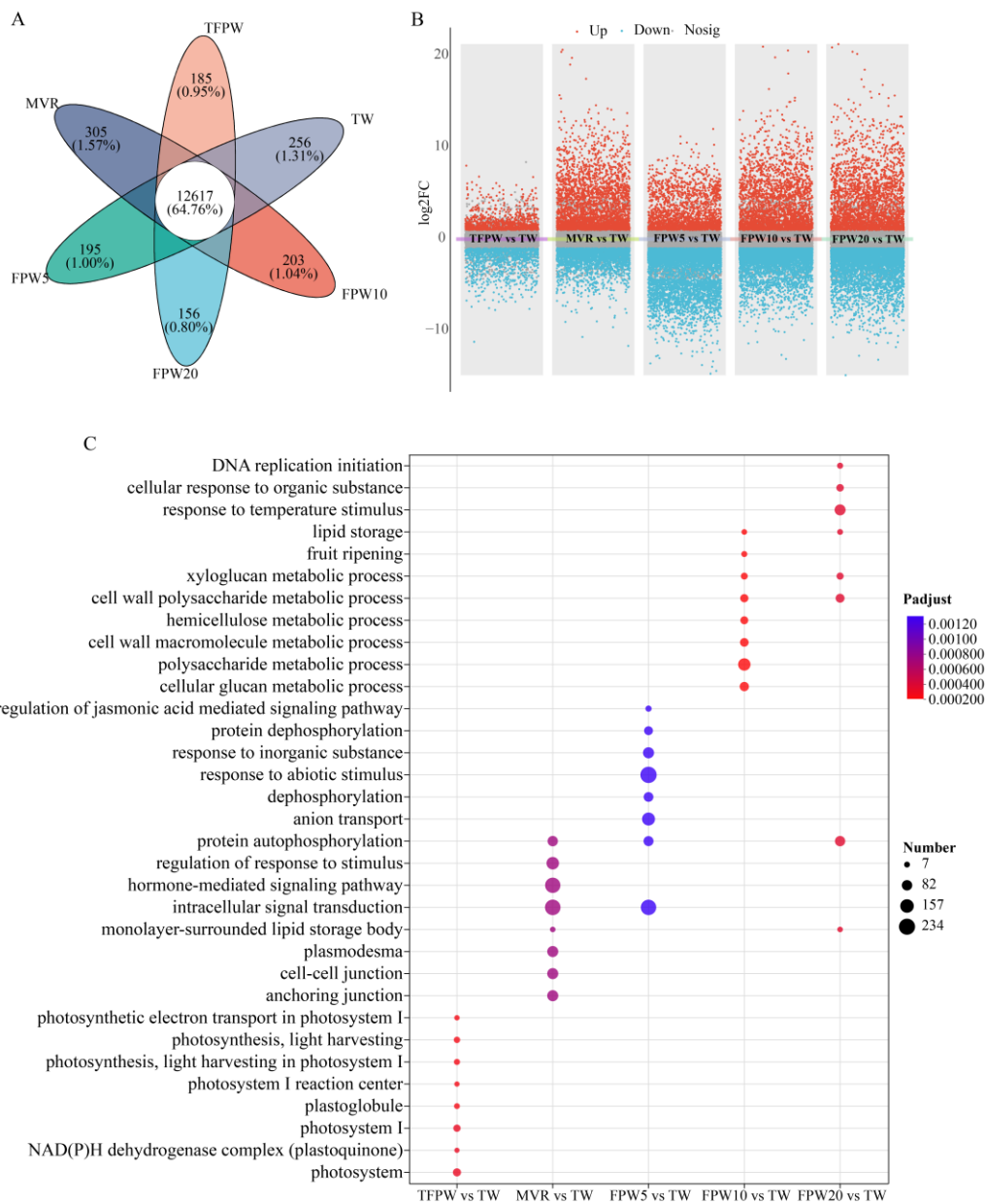


365
 366 **Fig 4.** Concentrations of Co, Cu, Cd, Pb, Ni, and Cr in various tissues of tomatoes
 367 irrigated with different solutions.

369 3.6. Effects of gene expression in tomato fruits

370 To better understand the effects of different treatments on the physiological basis

1 371 traits of tomatoes, fruits during the irrigation period were used as materials to analyze
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4 372 gene expression. The obtained RNA was free of pigments, proteins, sugars, and other
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6 373 impurities. Detailed sequencing quality control data are presented in **Tables S1 and S2**.
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9 374 Comparing the genes expressed in tomato fruits from different irrigation groups, a total
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11 375 of 25,299 expressed genes were detected in this analysis, of which 23,663 were known
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13 376 genes and 1,636 were new genes. A large number of co-expressed genes were identified
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15 377 in all the detected samples (12,617), accounting for 64.76% of the total detected genes
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17 378 (**Fig 5A**). DEGs, i.e., genes unique to each treatment group, may play an important role
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19 379 in producing differences in physiological characteristics. **Table S3** reports the number
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21 380 of genes that were up-regulated and down-regulated in the five experimental groups,
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23 381 compared to the control TW group. Specifically, the FPW20 group had the highest
24
25 382 number of DEGs (9108), FPW5 group had the highest number of down-regulated genes
26
27 383 (6257), MVR group had the highest number of up-regulated genes (3550). Key genes
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29 384 among up- and down-regulated ones may play important roles in tomato growth. To
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31 385 further understand and clarify the functional categories of differentially expressed genes,
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33 386 we used Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG)
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35 387 databases for enrichment analysis of the identified DEGs.
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390 **Fig 5.** (A) Venn diagram of all expressed genes in different types of irrigation groups.

391 The number shown in the outer non-overlapped portion indicates the number of genes

392 found in only one group, and the overlap of the circles represents the common number

393 of expressed genes. (B) Volcano plots of gene expression differences in TFPW vs. TW,

394 MVR vs. TW, FPW5 vs. TW, FPW10 vs. TW, and FPW20 vs. TW analyses. Gray dots

395 are not considered to be significantly differentially expressed. Red (up-regulated) and

1 396 green (down-regulated) points indicate DEGs ($|\log_2(\text{fold change})| > 1$, P adjust (FDR)
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4 397 < 0.05). (C) Bubble diagram of GO enrichment analysis of differentially expressed
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6 398 genes in tomato fruits (top 20 pathways based on enrichment factors).
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10 11 12 400 **3.6.1 GO enrichment analysis**

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15 401 Differential genes were analyzed for GO enrichment, and the top 8 enriched GO
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17
18 402 terms for each comparison group are presented in **Fig 5C**. At TFPW vs. TW, most of
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21 403 the significantly enriched GO terms were related to photosynthesis, such as
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23
24 404 photosystem, photosynthesis, and light harvesting. At MVR vs. TW, several key
25
26
27 405 biological processes and molecular functions in tomato fruits were enriched, primarily
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29
30 406 involving the regulation of response to stimuli, signal transduction pathways,
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33 407 modulation of cellular structures, and reorganization of nutrient metabolism. Under
34
35
36 408 high nitrogen conditions in MVR, tomatoes perceive and respond to environmental
37
38
39 409 changes through complex signaling pathways, regulating protein activity and enhancing
40
41
42 410 cell-to-cell interactions. In the high FPW treatment group (FPW5), some GO terms,
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44
45 411 such as response to inorganic substances, response to abiotic stimuli, and intracellular
46
47
48 412 signal transduction, were enriched, reflecting metabolic disturbances in the plants. The
49
50
51 413 plants initiated defensive mechanisms by regulating internal signaling pathways. In the
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53
54 414 medium and low FPW treatment groups (FPW10、FPW20), GO terms related to cell
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57 415 wall polysaccharide metabolic processes, cell wall macromolecule metabolic processes,
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60 416 and xyloglucan metabolic processes were significantly enriched, suggesting that

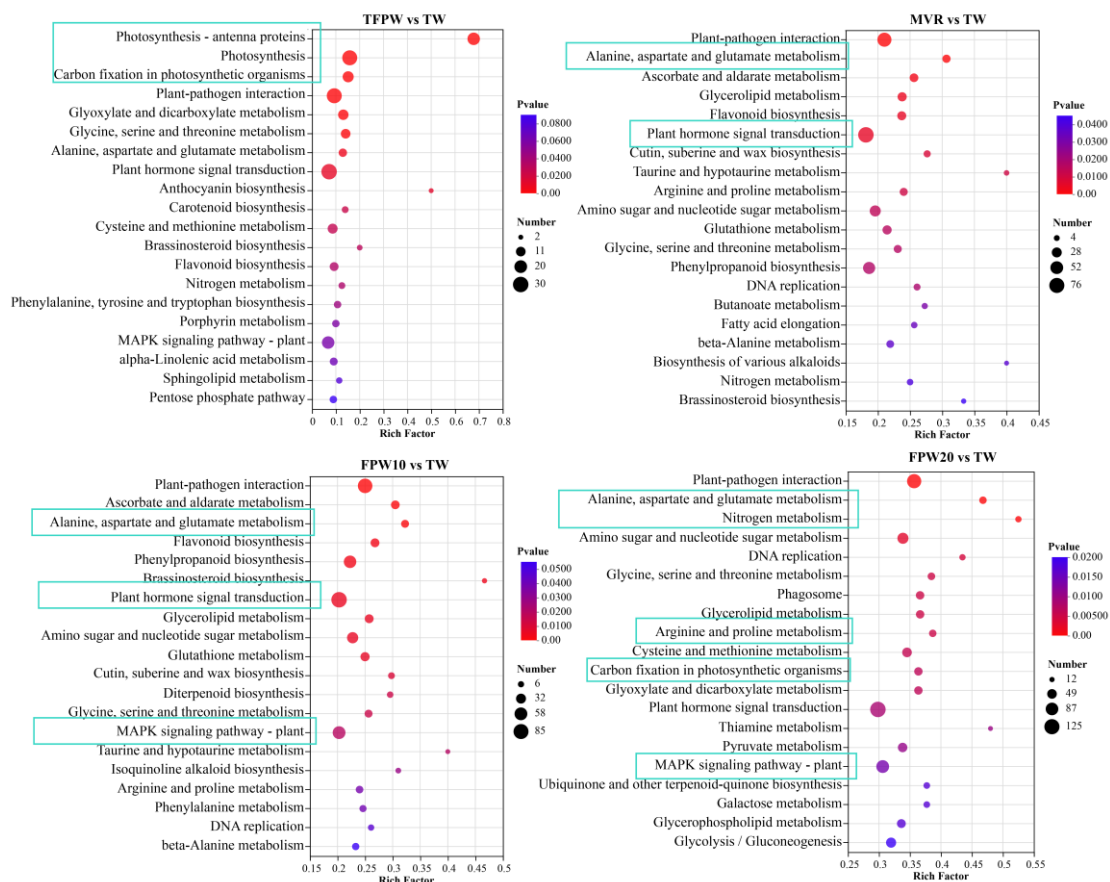
1 417 components in FPW may induce restructuring of cell structures and enhance cell wall
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4 418 stability to adapt to environmental stress.

5 6 419 **3.6.2 KEGG enrichment analysis**

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9 420 The KEGG enrichment results are shown in **Fig 6 and Fig S6**. At TFPW vs. TW,
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11
12 421 several pathways related to photosynthesis were enriched, such as Anthocyanin
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14
15 422 biosynthesis and Carotenoid biosynthesis. The expression of genes associated with light
16
17
18 423 capture and conversion in tomato fruits was significantly enhanced under TFPW
19
20
21 424 treatment. TFPW improved the photosynthetic efficiency of tomatoes, thereby
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23
24 425 promoting fruit growth and development, which is consistent with the GO enrichment
25
26
27 426 results of TFPW. In MVR and FPW treatments, DEGs were mostly enriched in
28
29
30 427 pathways related to amino acid metabolism, Plant hormone signal transduction, and
31
32
33 428 MAPK signaling pathway - plant. The enriched amino acid metabolism pathways
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36 429 primarily affect carbon and nitrogen metabolism in plants, such as Arginine and proline
37
38
39 430 metabolism, which influence chlorophyll synthesis. The enrichment of the Plant
40
41
42 431 hormone signal transduction pathway indicates that tomatoes undergo stress responses
43
44
45 432 to resist external influences. Carbon and nitrogen metabolism are the main pathways
46
47
48 433 for synthesizing carbohydrates and proteins, respectively, and play a crucial role in plant
49
50
51 434 growth and development. We hypothesize that MVR and FPW affect plant growth and
52
53
54 435 fruit yield by influencing carbon and nitrogen metabolic pathways.

55 436 To further analyze the impact of FPW on tomato carbon and nitrogen metabolism,
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57
58 437 we integrated the DEGs) from the three FPW groups and performed KEGG enrichment

1 438 analysis. The enriched amino acid-related metabolic pathways were Alanine, Aspartate,
 2
 3
 4 439 and Glutamate metabolism. Analyzing the pathway diagram (**Fig S7**), the upregulated
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 7 440 DEGs included Glutamate Pyruvate Transaminase (Soly03g123600), Asparaginase-
 8
 9
 10 441 like protein 1 (Soly06g069400, Soly04g078450, Soly04g078450), and Glutamate
 11
 12 442 Transporter 1 (Soly08g044270, Soly03g083440), while the downregulated DEGs
 13
 14
 15 443 included Asparagine synthetase (Soly04g055200), Nitrite Reductase 2
 16
 17
 18 444 (Soly07g041280), Nicotinate-nucleotide diphosphorylase (Soly12g014530), and
 19
 20
 21 445 Glutamine Synthetase (Soly01g080280). This suggests that FPW may negatively
 22
 23
 24 446 affect the metabolism of Alanine, Aspartate, and Glutamate in tomatoes.
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448
 449 **Fig 6.** KEGG pathway assignment of the DEGs in different comparison groups (top 20

1 450 pathways according to enrichment factor).

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5
6 452 **4. Conclusions**

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9 453 This work focused on the effects of shale gas FPW, treated shale gas FPW, and
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12 454 condensate from the mechanical vapor recompression system at the shale gas
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15 455 wastewater treatment plant on tomatoes when used for irrigating this crop under
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18 456 different scenarios. Specifically, morphological traits, metal and salt accumulation in
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20
21 457 various tissues, and fruit gene expression in tomatoes were analyzed. Compared to tap
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23
24 458 water, FPW in the intense leakage scenario (FPW5) and in the medium leakage scenario
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26
27 459 (FPW10) inhibited tomato growth to varying degrees, while tomato biomass increased
28
29
30 460 by 38% and 45% when treated FPW and when MVR solutions were used for irrigation,
31
32
33 461 respectively. The latter solutions also resulted in lower levels of toxic element
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35
36 462 accumulation. In addition, a large number of DEGs were induced to be down-regulated
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38
39 463 by FPW5, FPW10, and FPW20, which play important roles in carbon and nitrogen
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41
42 464 metabolism in tomato, and consequently affect fruit yield. In conclusion, the leakage of
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45 465 shale gas FPW may affect the safety of the soil and lead to the accumulation of toxic
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48 466 elements in plants, posing a potential danger to human health. On the other hand, the
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51 467 results suggest that appropriate treatment of FPW is a better choice compared to its
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54 468 dilution, in terms of soil and plant health, when such treated stream is reused for
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57 469 irrigation. In this sense, this study provides insights into the impact of different water
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60 470 qualities on tomato development, toxicological properties, and fruit gene expression,

1 471 but also guidance on the safe reuse strategy of shale gas FPW. Admittedly, this study
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4 472 has some limitations. Firstly, only one plant was studied in this paper. Secondly, FPW
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6 473 contains high concentrations of total dissolved solids, metals, organic matter, chemical
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9 474 additives, and natural radioactivity. This study evaluated the overall effects of FPW on
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11
12 475 the plant, and the toxicity of the individual components of FPW to the plant is unknown.
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15 476 In follow-up work, these issues will be explored in further experiments on a larger scale.
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21 478 **Supporting information**

23 479 The supporting information is available free of charge.
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29 481 **Acknowledgments**

32 482 This work was supported by the National Natural Science Foundation of China
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35 483 (52270075, 52070134, and 52300101), Sichuan University and Yibin City People's
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37
38 484 Government Strategic Cooperation Project (2020CDYB-2), and Cultivation Program
39
40
41 485 for Young and Middle-aged Leading Talents in Science and Technology (2023SCU17).
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44 486 A.T. acknowledges the support from the National Research Centre for Agricultural
45
46
47 487 Technologies (Agritech) funded by the Italian National Recovery and Resilience Plan
48
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50 488 (NRRP). The views and ideas expressed herein are solely those of the authors and do
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53 489 not represent the ideas of the funding agencies in any form.
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