

EFFECT OF TRANSPIRATION ON THE ACCUMULATION AND TRANSPORT OF BISPHENOL A IN CROP UNDER RECLAIMED WATER IRRIGATION

EFFET DE LA TRANSPIRATION SUR L'ACCUMULATION ET LE TRANSPORT DU BISPHENOL A DANS LES CULTURES IRRIGUEES AVEC DES EAUX RECYCLEES

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ABSTRACT

In light of the escalating water resource scarcity, the practice of reclaimed water irrigation has gained widespread adoption. Nevertheless, certain pollutants present in reclaimed water pose environmental health risks, particularly emerging contaminants for which the underlying mechanisms remain unclear. The role of transpiration, acting as the primary mechanism facilitating the uptake of organic pollutants by plants, and its impact on the fate of emerging pollutants within plants necessitate comprehensive exploration. An analysis was conducted of the relationship between transpiration and the concentrations of bisphenol A (BPA), bioconcentration factor (BCF), and translocation factor (TF) in each part of the pepper. Through regression analysis, significant correlations were identified between transpiration and the BPA concentration in the roots, leaves and stems of pepper, as well as significant correlations with the BCF, and TF in the stems, and leaves of the pepper. For the fruits and shoots of pepper, transpiration did not exhibit a significant correlation with BPA concentration, BCF, and TF. An in-depth elucidation of the impact of transpiration on the mechanisms underlying BPA accumulation and transport within colored peppers can contribute to informed decision-making regarding the agricultural reuse of reclaimed water, particularly in regions characterized by varying degrees of aridity.

Keywords: Reclaimed water irrigation, emerging contaminants; transpiration, accumulation and transport.

RÉSUMÉ

Face à la pénurie croissante des ressources en eau, la pratique de l'irrigation par eaux recyclées s'est largement généralisée. Néanmoins, certains polluants présents dans ces eaux posent des risques pour la santé environnementale, en particulier les contaminants émergents dont les mécanismes sous-jacents restent mal connus. Le rôle de la transpiration, qui constitue le principal mécanisme facilitant l'absorption des polluants organiques par les plantes, ainsi que son impact sur le devenir des contaminants émergents au sein des plantes, nécessite une étude approfondie. Une analyse a été menée sur la relation entre la transpiration et les concentrations de bisphénol A (BPA), le facteur de bioconcentration (FCB) et le facteur de translocation (FT) dans chaque partie du poivron. Grâce à une analyse de régression, des corrélations significatives ont été identifiées entre la transpiration et la concentration en BPA dans les racines, les feuilles et les tiges du poivron, ainsi que des corrélations significatives avec le BCF et le TF dans les tiges et les feuilles du poivron. En ce qui concerne les fruits

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et les pousses du poivron, la transpiration n'a pas montré de corrélation significative avec la concentration en BPA, le BCF et le TF. Une élucidation approfondie de l'impact de la transpiration sur les mécanismes sous-jacents à l'accumulation et au transport du BPA au sein des poivrons colorés peut contribuer à une prise de décision éclairée concernant la réutilisation agricole des eaux recyclées, en particulier dans les régions caractérisées par des degrés d'aridité variables.

Mots-clés : Irrigation par eaux recyclées; Contaminants émergents; Transpiration; Accumulation et transport.

1. INTRODUCTION

The reuse of non-conventional water sources, particularly reclaimed water, for agricultural irrigation has become an increasingly common practice in water-scarce regions. However, this practice introduces emerging contaminants into the soil–crop system, among which bisphenol A (BPA) is of particular concern. BPA is widely used as a monomer in the production of polycarbonate plastics and epoxy resins, and it is frequently detected in reclaimed water, untreated wastewater, and agricultural runoff (Tran et al., 2019). As a typical estrogenic endocrine disrupting chemical (e-EDC), BPA can elicit estrogenic responses at concentrations as low as ng/L to µg/L (Campbell et al., 2006; Minh et al., 2016). With the application of reclaimed water for irrigation, BPA can be taken up by plants, posing potential risks to human health through dietary exposure.

The accumulation and distribution of organic pollutants in plants are governed by multiple interrelated processes. Root uptake is influenced by the compound's physicochemical properties, while subsequent translocation to above-ground tissues is primarily driven by transpiration. However, the quantitative relationship between transpiration and contaminant translocation becomes substantially more complex under realistic soil conditions, where sorption, degradation, and plant-specific physiological traits interact simultaneously (Cañamera et al., 2016). Crop-specific differences further complicate this relationship. The ionization state of contaminants adds yet another layer of complexity—anionic species tend to accumulate preferentially in roots while showing limited translocation to leaves compared with neutral compounds (Dodgen et al., 2015). Despite these advances, a quantitative understanding of how transpiration dynamics over an entire reproductive cycle govern the organ-specific distribution of BPA in fruiting vegetables remains lacking.

Colored pepper is a widely cultivated, high-value vegetable crop that is consumed fresh, making the potential accumulation of contaminants in its edible fruits a direct dietary exposure concern. Its relatively long reproductive stage and substantial cumulative transpiration provide a relevant system for examining transpiration-driven contaminant transport. In the present study, the transpiration of colored peppers throughout their reproductive period were systematically documented. Subsequently, the concentration of BPA in various plant parts were measured post-harvest. Through comprehensive analysis, the accumulation and transfer of BPA in colored peppers and their relationship with transpiration were investigated. The implications of this research extend to serving as crucial references for the irrigation practices of farmland employing reclaimed water, especially in regions characterized by varying degrees of aridity.

2. MATERIALS AND METHODS

2.1 Test Area

The experiment was conducted between May 1, 2021, and May 10, 2022, at the Panggezhuang Experimental Station of the National Engineering and Technology Research Center for Water Saving Irrigation in Beijing (latitude 39°35'N, longitude 116°20'E). The experimental site within the solar greenhouse is located in the Daxing District in the southern part of Beijing, at an altitude of 30 m. The climate of the experimental station site is characterized by a warm temperate semi-humid continental monsoon climate. The average multi-year temperature is 11.6 °C, and the average multi-year rainfall of 556 mm. There is significant variation in precipitation across seasons, with a notable concentration

during the flood seasons from June to September, constituting approximately 80% of the annual precipitation. The annual average evapotranspiration is measured at 1,021 mm from the water surface and 450 mm from the land surface. The predominant soil types in the experimental area include powdery loam and sandy loam.

2.2 Materials

The soil employed in the potting test originated from the agricultural open space adjacent to the experimental station, specifically the 0-20 cm topsoil layer. The basic physicochemical properties of the soil were determined and are presented in Table 1. For the test crop, a common Solanaceous Vegetable, namely red pepper (variety: "Malda"), was selected. The stainless steel bucket utilized in the experiment had a diameter of 25 cm. The chemical fertilizer utilized in the study was Gold brand compound fertilizer (N-P₂O₅-K₂O: 15-15-15, total nutrients ≥ 45%), while the organic fertilizer employed was Beijing Woshengjie organic fertilizer (Organic matter ≥ 45%, N+P₂O₅+K₂O ≥ 5%).

Table 1. Basic physical and chemical properties of the test soil

Soil type	Particle size fraction (%)			pH	Field capacity	Organic matter	Total nitrogen	Available phosphorus	BPA
	Sand	Silt	Clay		(g/g)	(g/kg)	(g/kg)	(mg/kg)	(mg/kg)
Silty loam	28.75	65.00	6.25	8.37	0.27	14.6	0.763	63.6	0.0121

BPA standard (0.1 g, 100% purity, CAS: 80-05-7) and 4-n-NP standard (0.1 g, purity 99.0%, CAS: 104-40-5) was procured from Dr. Ehrenstorfer, Germany. Methanol (CAS: 67-56-1) and dichloromethane (CAS: 75-09-2) were of high-performance liquid chromatography (HPLC) grade and were procured from Honeywell, USA and Media Technology Inc., respectively. Anhydrous Na₂SO₄ (500 g, analytically pure, 99% purity, China National Pharmaceutical) underwent a 5-hour treatment in a muffle furnace at 500 °C before use. Non-quantitative, non-scaled glassware was subjected to a 4-hour heating process in an oven to eliminate any BPA residue. The ultrapure water for the experiment was generated using the UPW-10N series ultrapure water apparatus (resistivity 18.2 MΩ).

2.3 Experimental Design

The maximum BPA concentration was determined to be 2 µg/L based on the local reclaimed water effluent (Sun et al., 2010). To simulate soil conditions after 50 years of irrighexane ation, BPA was pre-added to the soil used in the test at a final concentration of 0.161 mg/kg, disregarding environmental processes such as soil adsorption degradation, soil-plant system interactions, and soil-water system migrations. To investigate the effects of transpiration on the enrichment and transport of BPA under different cropping patterns, an orthogonal experiment was conducted with treatments including lower limits of soil moisture (60%field capacity (FC), 70% FC, and 80% FC), fertilizer types (chemical fertilizer, organic fertilizer, and no fertilizer), and mulching methods (black film mulching, white film mulching, and no mulching). A total of 10 treatment combinations were established, each with four replicates. The experimental design is presented in Table 2.

Table 2. Treatment design for colored peppers

Treatment	Cropping patterns	Treatment	Cropping patterns
T1	60% FC+chemical fertilizer+white film mulching	T6	70% FC+no fertilizer+white film mulching
T2	60% FC+organic fertilizer+black film mulching	T7	80% FC+chemical fertilizer+no mulching
T3	60% FC+no fertilizer+no mulching	T8	80% FC+organic fertilizer+white film mulching
T4	70% FC+chemical fertilizer+black film mulching	T9	80% FC+no fertilizer+black film mulching
T5	70% FC+organic fertilizer+no mulching	T10	no crops+80% FC+no fertilizer+no mulching

Note: For the treatments receiving chemical fertilizer, or organic fertilizer, basal fertilizer was applied at a rate of 5 g of chemical fertilizer or 25 g of organic fertilizer per pot. During the flowering stage of colored pepper, topdressing was conducted twice, with each application consisting of 5 g of chemical fertilizer or 25 g of organic fertilizer per pot.

The cultivation of colored peppers commenced on May 12. Soil samples and plant samples were harvested after 126 days planting. To mitigate the impact of extraneous factors, the fruits of the treatments were harvested when a sufficient quantity had ripened. At the end of the experiment, collect soil and plant samples and calculate the transpiration throughout the entire reproductive period. The principal analytical and computational indexes comprised the BPA concentrations in soil and the BCF and TF in different tissues of colored peppers.

2.4 Analysis Method

2.4.1 Sample preservation

The soil samples were freeze-dried in batches with a freeze-dryer, and after the freeze-drying was completed, the soil samples were fully ground to ensure that there were no lumps, sieved through a 0.25 mm sieve to remove debris, packed into a sealed bag, and placed in a refrigerator at a constant temperature of -20 °C for storage. Rinse each plant sample with tap water, then rinse again with non-ionized water, absorb the surface moisture with filter paper, and then air dry until the surface is dry, and then weigh the fresh weight of plant samples; put the fresh plant samples into the oven, first kill the greening at 105 °C for 30 min, and then continue to dry at 80 °C until the constant weight, and then take out and dissipate the heat and then weigh the plant samples dry weight. The dried plant samples were ground to powder by a grinder, sealed with a ziplock bag and stored in a refrigerator at -20 °C. The concentrations of BPA in soil and plant samples were quantified by gas chromatography-mass spectrometry (GC 7890B-MS 5977B).

2.4.2 Pre-treatment of samples

Soil samples and plant samples were all preprocessed before the BPA was detected. 10 g of lyophilized soil sample was mixed uniformly after the surrogate (4-n-NP, 0.1 mg/kg) was added. The sample was then extracted using an ultrasonic extractor with a mixture containing 18 ml dichloromethane and 2 ml methanol for three times. The extract was centrifuged at 4000 r/min for 5 min, mixed, dehydrated with anhydrous sodium sulfate and then reduced to approximately 1.5 ml by rotary evaporator. The solution was subsequently filtered through an organic membrane with 0.22 µm pore size and further concentrated to 1 ml using a termovap sample concentrator. After the series of pretreatments, the concentrated extract could be stored at -20 °C for the following BPA analysis. The pretreatment of plant samples was similar with that of soil samples, except that the plant sample mass was 0.5 g.

2.4.3 GC-MS analysis method

Chromatographic conditions: A DB-5MS UI (30 m×0.25 µm×0.25 mm) column from Agilent was used for the on-line testing. The inlet and detector temperatures were set at 250 °C and 280 °C, respectively, and He was used as the carrier gas at a flow rate of 1 mL/min, and 1 µl of reaction solution was aspirated for non-shunt injection. Warming procedure: 50 °C (1 min) $\xrightarrow{5\text{ }^{\circ}\text{C}/\text{min}}$ 150 °C (1 min) $\xrightarrow{2\text{ }^{\circ}\text{C}/\text{min}}$ 180 °C (5 min) $\xrightarrow{12\text{ }^{\circ}\text{C}/\text{min}}$ 230 °C (1 min). Mass spectrometry conditions: the ion source was electron bombardment; the ion source temperature was 230 °C; the temperature of the quadrupole was 150 °C; the electron energy was 70 eV; the electron multiplier voltage was 1278 V; fragments from 50 to 300 amu were detected with both scan and sim mode.

Quality control and assurance: The limits of detection for the BPA was 15-33 ng/kg. The recoveries of BPA in soil were measured between 78.2% and 112.4% with the relative standard deviation coefficients (RSDs) ranging from 2.3% to 11.5%.

2.5 Data Analysis

2.5.1 Bioconcentration factor

BCF is defined as the ratio of BPA concentration in plant tissues to its residual concentration content in soil or planting solution. The parameter, to a certain extent, serves as an indicator of the migration capacity of BPA within the soil-plant system.

$$BCF_i = \frac{C_{pi}}{C_{soil}} \quad (1)$$

where C_{pi} is the concentration of BPA in plant tissue i (mg/kg), and C_{soil} is the weighted average BPA concentration in soil (mg/kg).

2.5.2 Translocation factor

TF is defined as the ratio of the BPA content in the shoot of plant tissues to its content in the root tissues. This parameter serves as an indicator of the ability of BPA to migrate and distribute among different tissues of plants.

$$TF_i = \frac{C_{pi}}{C_{root}} \quad (2)$$

where C_{pi} is the concentration of BPA in plant tissue i (mg/kg), and C_{root} is the concentration of BPA in root tissue (mg/kg).

2.5.3 Crop transpiration and transpiration efficiency

Crop transpiration and transpiration efficiency are computed according the following equations.

$$ET_1 = (G_{pot} + G_{dry\ soil} + G_{fresh\ weight} + I - G_{end}) \times \frac{1}{\rho_w} \times \frac{10^2}{0.3\ m \times 0.3\ m} \quad (3)$$

$$ET_2 = (G_{pot} + G_{dry\ soil} + I - G_{end}) \times \frac{1}{\rho_w} \times \frac{10^2}{0.3\ m \times 0.3\ m} \quad (4)$$

$$ET_3 = (ET_1 - ET_2) \quad (5)$$

where ET_1 is the transpiration of the mulch-treated crop (mm, the spacing between the rows of plants is 0.3m×0.3m), G_{pot} is the mass of empty stainless steel pot (kg), $G_{dry\ soil}$ is the mass of initial dry soil (kg), $G_{fresh\ weight}$ is the fresh weight of the crop after harvest (kg), I is the volume of crop irrigation during the experiment (kg), G_{end} is the mass of the whole potted plant at the end of the experiment without harvesting (kg), ρ_w is the water's density (kg/m³), ET_2 is the evaporation of blank control treatment (mm, the spacing between the rows of plants is 0.3m×0.3m), and ET_3 is the transpiration of the crop without mulching treatment (mm).

3 RESULTS AND DISCUSSION

3.1 Distribution of BPA in Colored Pepper Tissues

After harvest, varying concentrations of BPA were detected in all parts of the colored peppers. As depicted in Figure 1, the concentrations of BPA in roots, stems, leaves and fruits of the colored peppers were 104.3-366.6, 76.9-286.6, 170.0-578.5, and 58.1-377.3 ng/kg, respectively. The BPA concentrations in the shoots of the crop were 71.0-248.9 ng/kg. BPA exhibited higher concentrations in leaves, followed by roots. The log Kow value of BPA is 3.32 (Dodgen, 2014), indicating its moderate hydrophobicity. This property facilitates the uptake of BPA by plant roots and its subsequent translocation to above-ground tissues (Trapp and McFarlane, 1995).

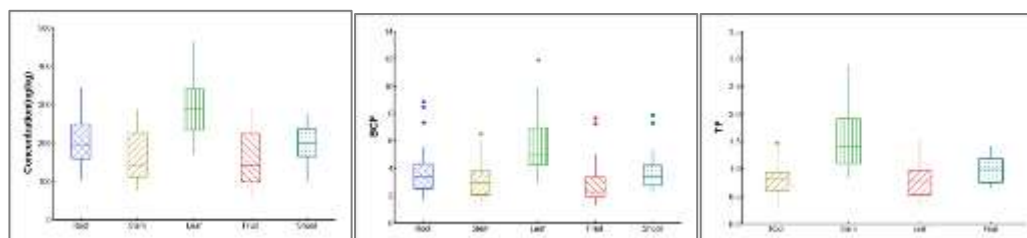


Figure 1. Concentration, accumulation and transport of BPA in crops

The leaves of colored pepper had the highest BCF (3.73-12.38), followed by roots (2.50-8.50), stems (1.95-4.92), and fruits (1.77-7.38); The shoot BCF ranged from 2.23 to 7.90. BPA showed strong enrichment in both leaves and roots, consistent with the behavior of neutral EDCs, for which shoot accumulation capacity is comparable to that of roots (Dodgen et al., 2015). The observed pattern of BCF being higher in leaves than in roots (BCF leaves > BCF roots) could be attributed to root activity and soil desorption which had promoted the metabolism of BPA within the roots. Furthermore, this experiment was sampled and tested at the end of the reproductive period. Compared to some previous short-term hydroponic experiments, the duration of crop incubation might in this experiment also affects migration and metabolism of BPA. The TF ranges of stem-root, leaf-root, fruit-root and shoot-root were 0.562-1.269, 1.236-2.056, 0.509-1.220 and 0.865-1.442, respectively. The ability of BPA to migrate from roots to leaves was significantly stronger than that to other tissues.

3.2 BPA Bioconcentration in Colored Pepper Tissues

Regression analysis of BPA concentration in different tissues of colored pepper with transpiration over the entire reproductive period revealed a significant correlation between BPA concentration in roots, stems and leaves of colored pepper and crop transpiration ($P < 0.05$). However, the BPA concentration in fruits and shoots exhibited a non-significant correlation ($P > 0.05$). This suggests that the uptake of BPA by colored pepper roots, stems and leaves is predominantly influenced by transpiration flow, a key factor in the plant uptake of many organic pollutants. Figure 2 illustrates that the BPA concentration in rhizome leaves did not consistently follow the increase in transpiration but exhibited a trend of initially increasing and then decreasing. This phenomenon may be attributed to the heightened metabolic activities in plant tissues when transpiration is higher.

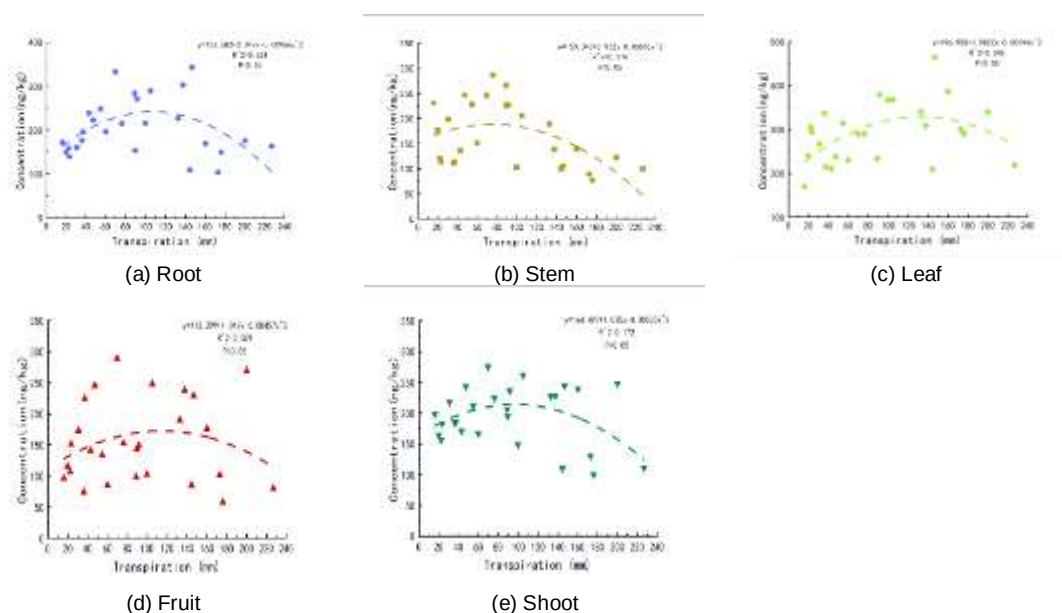


Figure 2. Regression between tissues concentration and transpiration

The uptake of BPA by fruits appears to be more influenced by various factors beyond transpiration. BPA, as a neutral substance, is predominantly transported in the xylem in crops, with a weaker ability to be transported to fruits through the phloem (Miller et al., 2016). Organic pollutants are deeply metabolized in the compartmentalization stage after functionalization and conjugation stages, and the metabolites enter the vesicles and cell walls (Kvesitadze et al., 2015), and fruits are rich in vesicles, which could potentially facilitate the metabolism of organic pollutants.

3.3 Effect of Transpiration on BPA Accumulation

BCF of stems and leaves was significantly correlated with transpiration ($P < 0.05$), whereas no significant correlation was observed for roots, fruits, or shoots ($P > 0.05$). The lack of correlation for roots may be attributed to the complex desorption and metabolic processes in the root zone, involving root exudates and rhizosphere microorganisms that participate in BPA degradation (Tong et al., 2021; Fu et al., 2022), thereby diminishing the direct influence of transpiration on root accumulation.

The accumulation of organic contaminants in fruits predominantly depends on phloem transport, and the transport potential of contaminants in both xylem and phloem is related to the inherent properties of these contaminants. Specifically, acidic substances in ionic compounds with a $pK_a < 7$ and $\log K_{ow} < 3$, which have a weak transmembrane capacity, are more prone to retention in the phloem and consequently in the fruit. It is noteworthy that BPA, characterized as weakly acidic, is primarily transported through the xylem in crops (Miller et al., 2016; Bromilow et al., 1990).

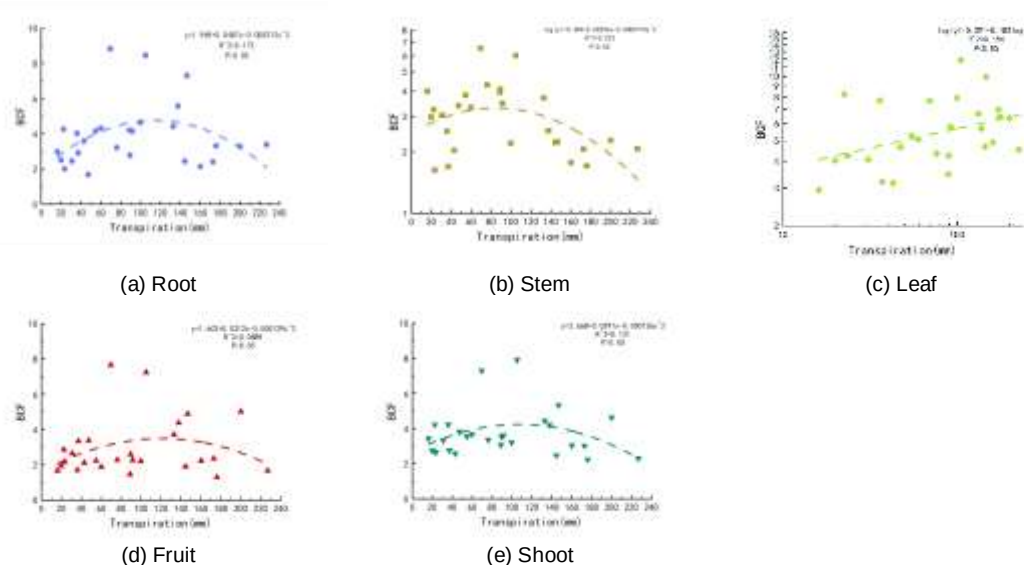


Figure 3. Regression between tissues BCF and transpiration

For crop stems, BPA, characterized by a small molecular mass and moderate lipophilicity, possesses the capability to traverse the Kjeldahl band into plant vascular bundles (Gong et al., 2020; Loffred et al., 2021). With the augmentation of transpiration, BPA tends to migrate more towards the leaves rather than accumulating significantly in the stems. BPA has a low volatility (Bagheri et al., 2021; Vasiljevic and Harner, 2021). Once BPA enters the leaves, it demonstrates limited tendency to volatilize, leading to its preferential accumulation within the leaf tissues.

3.4 Effect of Transpiration on BPA Transport

As shown in Figure 4, the TF of stems and leaves were significantly correlated with transpiration ($P < 0.05$), whereas no significant correlation was observed for fruits and shoots ($P > 0.05$). The translocation capacity of stems decreased with the increasing transpiration, which might be attributed

primarily to transpiration facilitating the upward movement of BPA through the xylem toward leaves. In contrast, the translocation capacity of leaves increased with rising transpiration. This pattern suggests that BPA transport is predominantly associated with the transpiration stream, with limited reverse movement from leaves to fruits, stems, and roots via the assimilative stream. Disparities in the metabolic capacity among different crop parts may influence translocation dynamics. In a previous investigation of BPA metabolism in water spinach, it was observed that BPA metabolites were concentrated in the roots, followed by the stems, while no BPA metabolites were detected in the leaves (Noureddin et al., 2004).

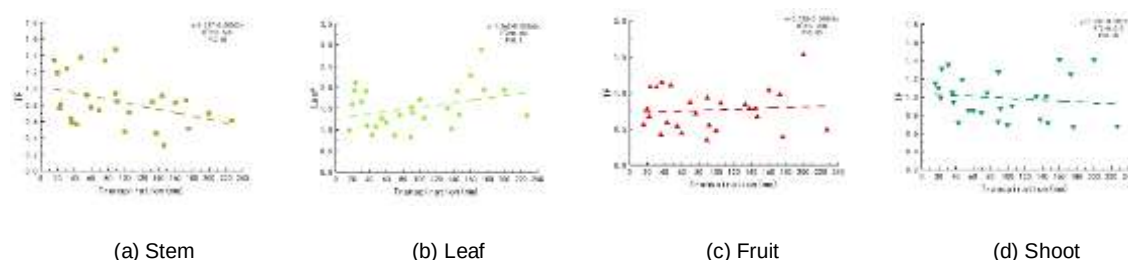


Figure 4. Regression between tissues TF and transpiration.

Apart from the intrinsic properties of the substance, the characteristics inherent to the crop itself also wield substantial influence. Organic contaminants are detected at low concentrations in the fruits of crops such as colored peppers, lettuce, and tomatoes (Lu et al., 2015). Conversely, in cucurbit zucchini and pumpkin, the contaminants exhibit pronounced accumulation and translocation in fruits. This phenomenon can be attributed to the presence of latex-like proteins, specifically Major Latex-like Proteins (MLPs), in some cucurbit plants. These MLPs have been identified as facilitators of the translocation of persistent organic pollutants to shoot (Fujita and Inui, 2021).

Furthermore, while stems and leaves exhibited a significant correlation with transpiration, the shoots did not maintain a significant correlation with transpiration. This discrepancy suggests that the accumulation and translocation behaviors of individual tissues differ substantially in both magnitude and underlying mechanisms. The findings highlight that the process of contaminant translocation in stems may be intricately linked to the environmental behavior of contaminants in various tissues. This aspect of correlation has been often overlooked in numerous short-term hydroponic studies. For higher plants, particularly as they enter the reproductive stage, there is a need for a nuanced and in-depth investigation of different tissues, rather than using the partial or partial tissue on the ground to replace the situation in each tissue.

4 CONCLUSIONS

The findings of this study revealed that, following prolonged irrigation with reclaimed water, a discernible concentration of BPA was present in the roots, stems, leaves, and fruits of colored peppers. Notably, leaves exhibited a more pronounced enrichment and translocation capacity compared to other plant parts. Transpiration exerted a significant influence on both the concentration and translocation capacity of BPA in stems and leaves. In contrast, its impact was limited to the concentration of BPA in the roots of colored peppers. The environmental behavior of BPA in fruits and shoots exhibited no significant correlation with transpiration. The accumulation and translocation of BPA, as a neutral substance in plants, were predominantly associated with xylem transport, with phloem transport playing a comparatively weaker role. Consequently, with increasing transpiration, BPA demonstrated a propensity to migrate from stems to leaves, while its accumulation in fruits was comparatively weaker. Given the constrained direct impact of transpiration on the environmental dynamics of pollutants in crops, it is advisable to redirect focus towards investigating the indirect effects in subsequent research endeavors. Specifically, there is a pressing need to quantify and delve into the role of plant metabolism in influencing pollutant behavior. The intricate association between the source and destination of BPA within stems

and other plant parts warrants in-depth exploration. Furthermore, it is imperative to unravel the mechanisms underlying the variations in enrichment and translocation characteristics of pollutants across different plant parts.

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