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The Impact of Shale Oil Residue on the Growth and Physiological Characteristics of Corn Seedlings under Saline Soil Conditions

Jian Huang [1,2,†], Zhiduo Dong [3,†], Ajing Meng [1], Tong Qi [1,2,*] and Chunhui Ge [1,2]

[1] Institute of Soil Fertilizer, Agricultural Water Saving, Xinjiang Academy of Agricultural Sciences, Urumqi 830091, China; huangjian@xaas.ac.cn (J.H.); maj19890917@163.com (A.M.); gechunhui@xaas.ac.cn (C.G.)
[2] Key Laboratory of Saline-Alkali Soil Improvement and Utilization (Saline-Alkali Land in Arid and Semi-Arid Regions), Ministry of Agriculture and Rural Affairs, Urumqi 830091, China
[3] College of Water Conservancy and Civil Engineering, Xinjiang Agricultural University, Urumqi 830052, China; dzd1281228561@163.com
* Correspondence: zxb2023321005@cau.edu.cn
† These two authors contributed equally.

Abstract: Soil salinization is a primary environmental factor leading to reduced crop yields, and oil shale waste residues may have the potential to alleviate plant salt stress. This study aims to investigate the effects of three types of oil shale waste residues (fine concentrate ore, fine ore, and semi-coke) on the growth and physiological characteristics of maize seedlings in saline–alkali soil. The results indicate the following: (1) All three types of oil shale waste residues increased the root vitality of seedlings and reduced the root proline content. (2) The three types of oil shale waste residues increased the activity of superoxide dismutase (1.70% to 97.19%) and peroxidase (29.39% to 61.21%) in maize seedlings, but there were differences in their effects on catalase activity. The fine ore and semi-coke treatments increased catalase activity (4.98% to 77.42%), while fine concentrate ore decreased catalase activity (39.28% to 5.30%). (3) The three types of oil shale waste residues effectively alleviated the degree of membrane lipid peroxidation in maize seedling leaves. (4) Principal component analysis showed that the semi-coke treatment was beneficial to the growth and physiology of maize seedlings in saline–alkali soil, with the optimal effect occurring at a 0.2% addition rate. In conclusion, adding semi-coke to saline–alkali soil promotes the growth of maize by regulating its physiological and biochemical mechanisms, alleviating the salt stress on maize seedlings caused by salt content.

Keywords: shale oil residue; maize; antioxidant enzyme activity; salt stress

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1. Introduction

With the growing global population and food demand, soil salinization poses a serious challenge to agricultural production [1–3]. In China, a quarter of the cultivated land for corn is affected by salt stress [4]. China is the world's largest producer of unconventional shale oil, with Xinjiang possessing abundant shale oil resources, particularly in the crucial exploration area of Jimusaer County [5–7]. However, the development of shale resources generates a substantial amount of waste, constituting 60–80% of the total, posing significant challenges in environmental and resource management [8]. In this context, research on the potential feasibility of converting shale oil waste into reusable agricultural materials has emerged.

Excessive soil salinity makes it difficult for plant roots to absorb water, leading to the uptake of harmful ions by crops [1,2]. This triggers issues such as osmotic stress and ion toxicity, severely disrupting normal plant growth and metabolic processes [9]. Salt stress causes an imbalance in the oxidative–reductive state within plant cells, accelerating the generation of reactive oxygen species (ROS) [10,11]. The excessive production of ROS

damages cell membranes, causing oxidative injury to plant tissues, and even resulting in plant death. The survival of plants under salt stress depends, to a large extent, on the capability of their antioxidant systems, which play a crucial role in mitigating damage caused by ROS [12].

Our primary objective is to address the challenge of recycling substantial industrial waste generated during oil shale production and processing. We aim to repurpose this waste as a material for enhancing saline–alkali conditions in agricultural production. This study delves into the feasibility and safety of this approach, seeking to contribute a novel solution to efforts to solve the global food security issue. The utilization of shale oil waste as a soil conditioner, stabilizer, and fertilizer holds the potential to enhance soil physical, chemical, and biological properties, leading to improved crop yields [13]. Shale oil waste is rich in micronutrients, oxygen-containing functional groups, organic matter, and acidic and alkaline oxides, exhibiting excellent water retention and nutrient retention capabilities. With its porous structure and high specific surface area [13–17], shale oil waste presents promising prospects for ameliorating low-yielding saline–alkali land. While shale oil waste has the potential to play a crucial role in improving agricultural soil, the precise mechanisms underlying its effectiveness remain unclear. Hence, this study collected three types of shale oil waste from the Baoming mining area in Jimusaer County, Xinjiang, namely fine concentrate ore, fine ore, and semi-coke. Our objective is to observe the impact of adding various proportions of shale oil waste to saline–alkali soil as a cultivation substrate on the growth, physiological, and biochemical indicators of maize seedlings. By comprehensively evaluating the overall effects of each treatment on maize seedlings in saline–alkali soil through principal component analysis, we aim to identify the specific type of shale oil waste that can mitigate salt damage and elucidate its mechanisms in alleviating salt stress in plants.

2. Materials and Methods

2.1. Experimental Materials

Oil shale waste residues, including fine concentrate ore (ore with particle size less than 6 mm after desliming and dewatering), fine ore (raw ore broken into particles of less than 6 mm), and semi-coke (the remaining raw ore left after oil shale distillation containing a small amount of residual carbon, similar to the raw material of volcanic ash, and the removal of volatiles, carbon, or other organic acids during the distillation or combustion process, forming a porous structure), were sourced from the Baoming mining Area in Jimusaer County, Xinjiang, China. The cultivation substrate used was typical saline–alkali desert soil in Xinjiang, characterized by sulfate-chloride salinity and classified as moderately saline soil. The maize variety used in this study was "Jiushenghe 2468", with seeds selected for their fullness, uniform size, and intact embryos.

The basic physicochemical properties of the saline–alkali soil and oil shale waste residues are presented in Table 1. The heavy metal content in both was found to be below the limits specified in the People's Republic of China National Standard [18].

Table 1. Basic physicochemical properties of the experimental materials.

Materials	Unit	Cultivation Substrate	Oil Shale Waste Residue		
		Saline–Alkali Soil	Fine Concentrate Ore	Fine Ore	Semi-Coke
pH	-	8.55	8.26	7.89	8.43
Water-soluble salts	$g \cdot kg^{-1}$	9.4	4.2	7.5	8.2
Total nitrogen	$g \cdot kg^{-1}$	0.4	2.57	0.93	2.73
Organic matter	%	4.31	28.08	6.9	13.26
Hydrolysable nitrogen	$mg \cdot kg^{-1}$	22.5	55.5	55.5	69.1
Available phosphorus	$mg \cdot kg^{-1}$	7.9	44.3	42.8	52.9
Quick-acting potassium	$mg \cdot kg^{-1}$	114	72	85	105
Arsenic	$mg \cdot kg^{-1}$	<0.002	<0.002	<0.002	<0.002

Table 1. *Cont.*

Materials	Unit	Cultivation Substrate	Oil Shale Waste Residue		
		Saline–Alkali Soil	Fine Concentrate Ore	Fine Ore	Semi-Coke
Mercury	$mg \cdot kg^{-1}$	<0.002	0.044	0.028	0.041
Chromium	$mg \cdot kg^{-1}$	<0.04	0.7	0.6	0.7
Lead	$mg \cdot kg^{-1}$	9	38	36	30
Cadmium	$mg \cdot kg^{-1}$	<0.01	<0.01	<0.01	<0.01
Nickel	$mg \cdot kg^{-1}$	12	66	68	66

2.2. Experimental Design

The experiment was conducted using potted plants in the artificial climate chamber at the Agricultural Mechanization Research Institute of Xinjiang Academy of Agricultural Sciences (longitude 87.57867, latitude 43.81211). The laboratory maintained a temperature range of 18 to 28 °C, a relative humidity of 60%, and utilized natural light with a transmittance exceeding 80%. Saline–alkali soil and oil shale waste residue were separately sieved through a 2 mm mesh and reserved for use. The experimental design included 4 (addition levels) × 3 (types of oil shale waste residue) treatments, along with one blank control. Each treatment had 3 replicates, resulting in a total of 39 pots. The four addition levels were 0.1%, 0.2%, 0.4%, and 0.8%. Saline–alkali soil and the respective treatment materials were proportionally loaded into round plastic pots with a diameter of 17 cm and a height of 13 cm. Each pot was filled with 3 kg of substrate. After filling, 450 mL of distilled water was added, and the pots were allowed to stand for one day before sowing. On 6 June 2022, ten maize seeds were sown in each pot and covered with plastic wrap. After seedlings emerged, the plastic wrap was removed. Seven days after emergence, 5~6 corn seedlings with consistent growth were planted in each pot. Uniform applications of urea (75 kg/ha), ammonium dihydrogen phosphate (75 kg/ha), and potassium sulfate (60 kg/ha) were carried out in two split doses during the seedling emergence and jointing stages. The experiment was conducted from 6 June 2022 to 29 July 2022, spanning a period of 54 days.

2.3. Index Determination and Methods

2.3.1. Growth Index Determination

Seedling height was measured directly using a tape measure. On 6 July 2022, the height of each seedling in each treatment was measured from the base of the seedling to its highest point using a tape measure [19], and calipers were used to measure stem width at the base [19]. On 29 July 2022, destructive sampling was carried out. We selected 3 corn seedlings from each treatment, uprooted them, and washed them with distilled water to remove the soil adhered to the roots. The aboveground parts (stems and leaves) and underground parts (roots) were separated, and their fresh weights were measured using an electronic scale. Samples were placed in paper bags and dried in an oven at 105 °C for 30 min, followed by drying at 75 °C to a constant weight. The dry weights were then measured. The root/shoot ratio and seedling vigor index were calculated according to the following formulas [19,20].

$$\text{Root/Shoot ratio} = \left(\frac{\text{Underground dry weight}}{\text{Aboveground dry weight}}\right) \quad (1)$$

$$\text{Seedling vigor index} = \left(\frac{\text{Stem width}}{\text{height}} + \frac{\text{Underground dry weight}}{\text{Aboveground dry weight}}\right) \times \text{Total dry weight} \quad (2)$$

2.3.2. Physiological Index Measurement

The determination of root vitality was conducted using the chlorotriphenyltetrazolium chloride colorimetric method [21]. proline (PRO) was measured through the absorbance

method [22]. The determination of malondialdehyde (MDA) content was carried out using the thiobarbituric acid method [19].

To determine enzyme activity, we needed to obtain clean leaf samples and immediately cool them with liquid nitrogen after sampling. After cooling, the samples were stored in a −80 °C freezer for later use. In the analysis of antioxidant enzymes, we used 0.5 g of maize seedling leaves. First, fresh leaf tissues were ground in a cold water bath and mixed with pH 7.4, 0.05 mol/L phosphate-buffered saline. Subsequently, the supernatant, obtained via centrifugation at 8000× *g* for 10 min at 4 °C, was used for the subsequent analysis of antioxidant enzyme activity. Superoxide dismutase (SOD) activity was determined using the nitroblue tetrazolium (NBT) photoreduction method [23]. Additionally, peroxidase (POD) activity was determined using the guaiacol method [24]. Catalase (CAT) activity was measured using the ultraviolet absorption method [23]. To determine the activity of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), we utilized specific assay kits, namely SOD-1-W, POD-1-Y, and CAT-1-Y, respectively. These assay kits were provided by Suzhou Keming Biotechnology Co., Ltd. (Suzhou, China).

2.4. Statistical Analysis

The obtained data are expressed as mean ± standard deviation. Experimental data management was carried out using Excel 2016. Single-factor analysis of variance (ANOVA) was performed using SPSS 26.0, followed by Duncan's post hoc test to determine the significance of differences ($p < 0.05$ is considered significant). Two-way analysis of variance was conducted using SPSS 26.0, and Tukey's post hoc multiple comparison test was applied to compare mean differences ($p < 0.05$ is considered significant). Principal component synthesis was performed using SPSS 26.0. Bar charts were created using Origin 2018. Hierarchical clustering and principal component analysis (PCA) were carried out using RStudio. The first two principal components (PC1 and PC2) were extracted. The plotting process involved the use of the "pheatmap" and "devtools" packages.

3. Results

3.1. Growth Characteristics

According to Table 2, it can be observed that the treatments with fine concentrate ore (Y), fine ore (M), and semi-coke (B) did not significantly affect seedling height and root/shoot ratio ($p > 0.05$). However, they had a significant impact on stem width, fresh and dry weight, and seedling vigor index ($p < 0.05$). In terms of stem width, all three treatments showed a decrease compared to CK. For underground fresh weight and dry weight, the addition of 0.2% Y, 0.2% M, and 0.1~0.8% B treatments increased compared to CK. In aboveground fresh weight and dry weight, the 0.4% M and the 0.1~0.8% B treatments increased compared to CK. For total fresh weight and dry weight, the 0.4% M and the 0.1~0.8% B treatments increased compared to CK. In terms of seedling vigor index, the 0.1~0.8% B treatments increased compared to CK, with significant differences observed at the 0.2% addition level.

A dual-axis plot from the principal component analysis revealed the relationships between various treatments and indicators (Figure 1). Principal Component 1 explained 62.7% of all variables, while Principal Component 2 explained 19.2%, collectively explaining 81.9% of the variation. Principal Component 1 divided the treatments into two groups, with 0.8% B, 0.4% M, 0.1% B, 0.4% B, 0.2% B, and 0.2% Y treatments on the left, and CK, 0.8% M, 0.4% Y, 0.8% Y, 0.1% M, 0.2% M, and 0.1% Y treatments on the right. Principal Component 2 separated the growth indicators into two groups, with stem width (X2), aboveground dry weight (X6), height (X1), aboveground fresh weight (X5), total fresh weight (X7), and total dry weight (X8) at the bottom, and seedling vigor index (X10), underground dry weight (X4), underground fresh weight (X3), and root/shoot ratio (X9) at the top.

Table 2. Effects of oil shale waste residues on seedling growth characteristics.

Addition Levels	Treatment	Height (cm)	Stem Width (mm)	Fresh Weight (g/plant)			Dry Weight (g/plant)			Root/Shoot Ratio	Seedling Vigor Index
				Underground	Aboveground	Total	Underground	Aboveground	Total		
0	CK	41.78 ± 0.59 a	4.85 ± 0.16 a	0.80 ± 0.18 ab	6.68 ± 0.42 ab	7.48 ± 0.60 abc	0.10 ± 0.02 b	0.98 ± 0.04 ab	1.07 ± 0.05 b	0.10 ± 0.02 a	0.23 ± 0.02 b
0.1%	Y	41.22 ± 1.84 a	4.33 ± 0.23 bc	0.62 ± 0.24 b	4.57 ± 1.03 b	5.19 ± 1.14 c	0.09 ± 0.03 b	0.72 ± 0.11 b	0.81 ± 0.10 b	0.13 ± 0.05 a	0.19 ± 0.03 b
	M	39.06 ± 2.56 a	4.23 ± 0.46 bc	0.93 ± 0.62 ab	6.21 ± 2.41 ab	7.14 ± 2.71 abc	0.09 ± 0.07 b	0.85 ± 0.28 ab	0.94 ± 0.32 b	0.10 ± 0.09 a	0.20 ± 0.11 b
	B	44.59 ± 4.03 a	4.49 ± 0.36 abc	0.94 ± 0.14 ab	7.58 ± 0.25 a	8.52 ± 0.27 ab	0.14 ± 0.02 ab	1.11 ± 0.06 a	1.25 ± 0.05 ab	0.13 ± 0.02 a	0.28 ± 0.04 b
0.2%	Y	40.39 ± 2.78 a	4.32 ± 0.20 bc	1.24 ± 0.46 a	6.44 ± 1.76 ab	7.67 ± 2.07 abc	0.16 ± 0.06 ab	0.86 ± 0.24 ab	1.02 ± 0.29 b	0.18 ± 0.04 a	0.30 ± 0.10 b
	M	39.56 ± 4.06 a	4.33 ± 0.27 bc	0.94 ± 0.11 ab	6.24 ± 0.92 ab	7.18 ± 1.02 abc	0.13 ± 0.01 b	0.90 ± 0.12 ab	1.02 ± 0.12 b	0.14 ± 0.02 a	0.26 ± 0.02 b
	B	43.52 ± 2.78 a	4.39 ± 0.29 abc	1.02 ± 0.40 ab	8.37 ± 1.59 a	9.39 ± 1.67 a	0.34 ± 0.38 a	1.21 ± 0.26 a	1.55 ± 0.59 a	0.26 ± 0.26 a	0.65 ± 0.69 a
0.4%	Y	42.31 ± 4.64 a	4.38 ± 0.11 abc	0.55 ± 0.23 b	6.21 ± 1.96 ab	6.77 ± 1.82 abc	0.08 ± 0.03 b	0.91 ± 0.33 ab	0.99 ± 0.32 b	0.11 ± 0.07 a	0.19 ± 0.03 b
	M	41.17 ± 1.32 a	4.49 ± 0.18 abc	0.77 ± 0.26 ab	7.96 ± 1.67 a	8.74 ± 1.71 ab	0.11 ± 0.03 b	1.15 ± 0.23 a	1.26 ± 0.22 ab	0.10 ± 0.04 a	0.26 ± 0.03 b
	B	44.34 ± 2.54 a	4.61 ± 0.08 abc	1.23 ± 0.27 a	7.45 ± 1.06 a	8.68 ± 0.79 ab	0.16 ± 0.04 ab	1.05 ± 0.13 ab	1.21 ± 0.09 ab	0.16 ± 0.07 a	0.31 ± 0.05 b
0.8%	Y	39.44 ± 1.58 a	4.36 ± 0.13 bc	0.77 ± 0.11 ab	5.75 ± 1.17 ab	6.52 ± 1.28 bc	0.11 ± 0.02 b	0.87 ± 0.13 ab	0.98 ± 0.15 b	0.12 ± 0.01 a	0.23 ± 0.03 b
	M	39.17 ± 3.47 a	4.75 ± 0.09 ab	0.64 ± 0.15 b	5.77 ± 0.91 ab	6.41 ± 0.99 bc	0.09 ± 0.01 b	0.89 ± 0.12 ab	0.98 ± 0.12 b	0.10 ± 0.02 a	0.22 ± 0.01 b
	B	42.76 ± 2.12 a	4.61 ± 0.28 abc	0.85 ± 0.10 ab	7.61 ± 0.90 a	8.46 ± 0.97 ab	0.12 ± 0.02 b	1.13 ± 0.17 a	1.25 ± 0.18 ab	0.11 ± 0.02 a	0.27 ± 0.04 b

Note: different lowercase letters indicate significant differences at the 0.05 probability level ($p < 0.05$), determined by one-way analysis of variance (ANOVA) and Duncan's post hoc test. The data are presented as means ± standard deviation (SD) calculated from three repetitions. Y: fine concentrate ore, M: fine ore, B: semi-coke.

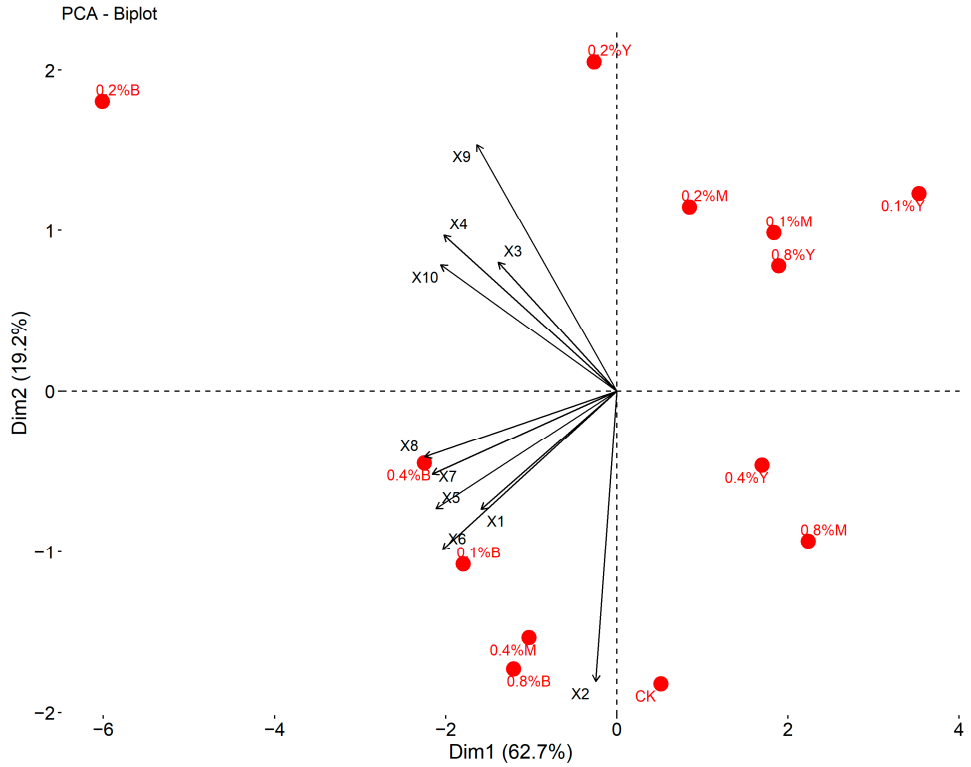

Figure 1. Biplots based on principal component analysis (PCA), illustrating the relationships between maize growth indicators and applied treatments. X1: height, X2: stem width, X3: underground fresh weight, X4: underground dry weight, X5: aboveground fresh weight, X6: aboveground dry weight, X7: total fresh weight, X8: total dry weight, X9: root/shoot ratio, X10: seedling vigor index. Y: fine concentrate ore, M: fine ore, B: semi-coke. 0.1%, 0.2%, 0.4%, and 0.8%, respectively, represent volume fraction additions of 0.1%, 0.2%, 0.4%, and 0.8% in the cultivation substrate.

3.2. Root Vitality

The effect of fine concentrate ore (Y), fine ore (M), and semi-coke (B) treatments on the root vitality of maize seedlings is depicted in Figure 2. Compared to the control (CK), treatments with Y, M, and B resulted in an enhancement in root system vitality. The respective increases ranged from 4.53% to 40.73%, 3.99% to 22.35%, and −2.31% to 74.37%. However, there were isolated cases, notably the 0.1% B treatment, which exhibited a decrease compared to CK, though the difference was not statistically significant.

3.3. Proline Content

The impact of treatments with fine concentrate ore (Y), fine ore (M), and semi-coke (B) on the proline content in the roots of maize seedlings is illustrated in Figure 3. Except for the 0.4% Y, 0.2% M, and 0.8% B treatments, which showed an increase compared to CK, all other treatments significantly decreased proline content compared to CK. Among them, the Y treatment exhibited the greatest reduction at the 0.1% addition level, with a decrease of 64.62%. The M treatment showed the maximum reduction at the 0.8% addition level, with a decrease of 68.62%. The B treatment had the most substantial reduction at the 0.2% addition level, with a decrease of 64.25%.

3.4. Malondialdehyde Content

Compared to CK, treatments with fine concentrate ore (Y), fine ore (M), and semi-coke (B) all resulted in a reduction in the malondialdehyde content in maize seedling leaves (Figure 4). Only the 0.4% B and 0.8% B treatments exhibited no significant difference compared to CK. Among these treatments, the Y treatment showed the greatest reduction, with a decrease of 16.96% at the 0.4% addition level. The M treatment exhibited the

maximum reduction, reaching 27.73%, at the 0.1% addition level. The B treatment had the most substantial reduction, at 26.49%, at the 0.2% addition level.

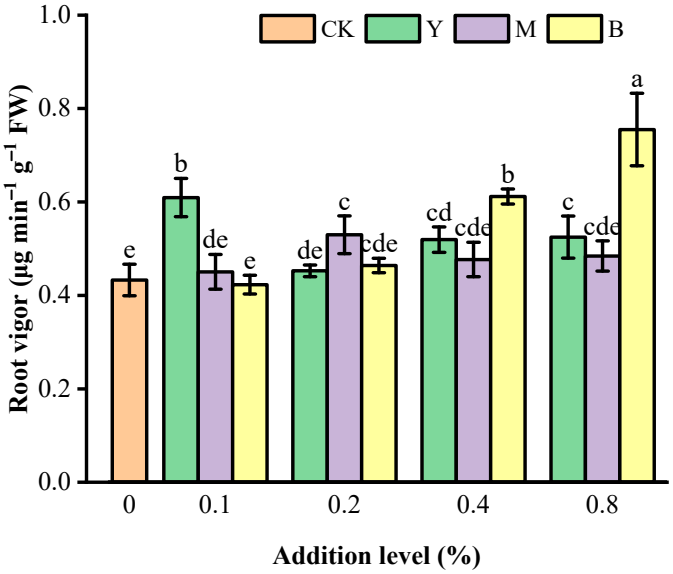

Figure 2. Effects of oil shale waste residues on the root vitality of maize seedlings. Different lowercase letters indicate significant differences at the 0.05 probability level ($p < 0.05$), determined by one-way analysis of variance (ANOVA) and Duncan's post hoc test for significance. The vertical bar chart represents the mean ± standard deviation (SD) calculated from three repetitions. Y: fine concentrate ore, M: fine ore, B: semi-coke.

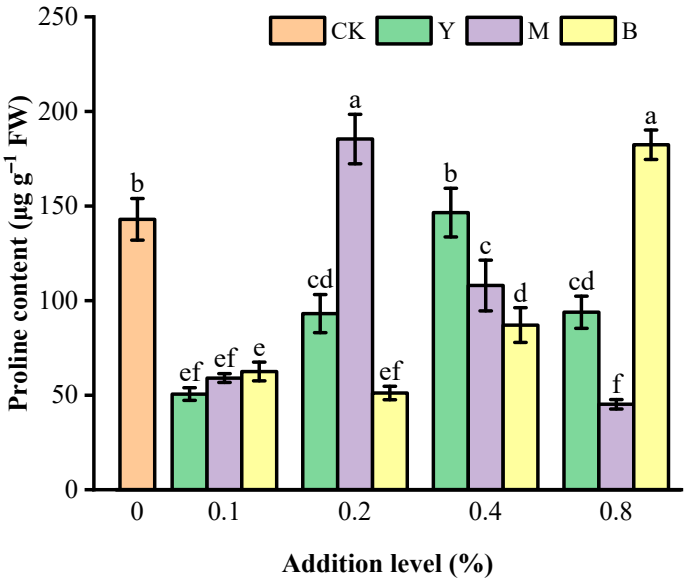

Figure 3. Effects of oil shale waste residues on the proline content in the root system of maize seedlings. Different lowercase letters indicate significant differences at the 0.05 probability level ($p < 0.05$), determined by one-way analysis of variance (ANOVA) and Duncan's post hoc test for significance. The vertical bar chart represents the mean ± standard deviation (SD) calculated from three repetitions. Y: fine concentrate ore, M: fine ore, B: semi-coke.

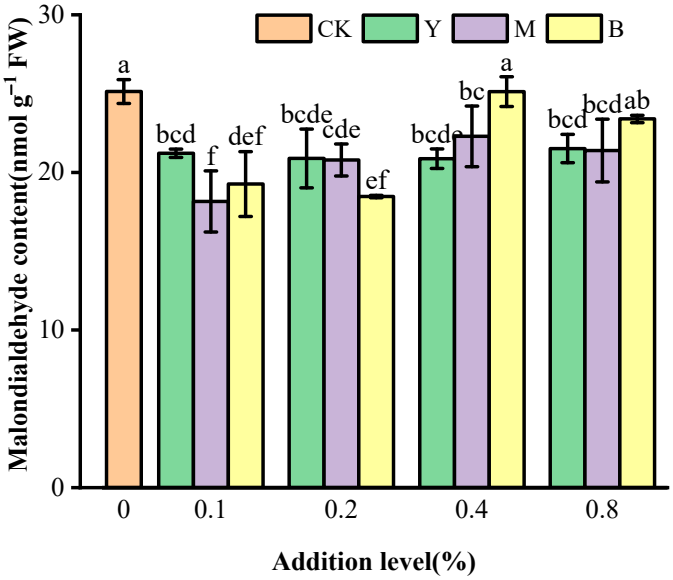

Figure 4. Effects of oil shale waste residues on the malondialdehyde content in maize seedling leaves. Different lowercase letters indicate significant differences at the 0.05 probability level ($p < 0.05$), determined by one-way analysis of variance (ANOVA) and Duncan's post hoc test for significance. The vertical bar chart represents the mean ± standard deviation (SD) calculated from three repetitions. Y: fine concentrate ore, M: fine ore, B: semi-coke.

3.5. Antioxidant Enzyme Activity

The impact of treatments with fine concentrate ore (Y), fine ore (M), and semi-coke (B) on the antioxidant enzyme activity in maize seedling leaves is illustrated in Figure 5. Compared to CK, all three types of oil shale waste residues increased the activity of superoxide dismutase (SOD), particularly the Y treatment, with an increase ranging from 88.11% to 97.19%. Treatments with Y, M, and B significantly enhanced the activity of peroxidase (POD), with increases ranging from 37.45% to 51.91%, 37.67% to 61.21%, and 29.39% to 44.17%, respectively.

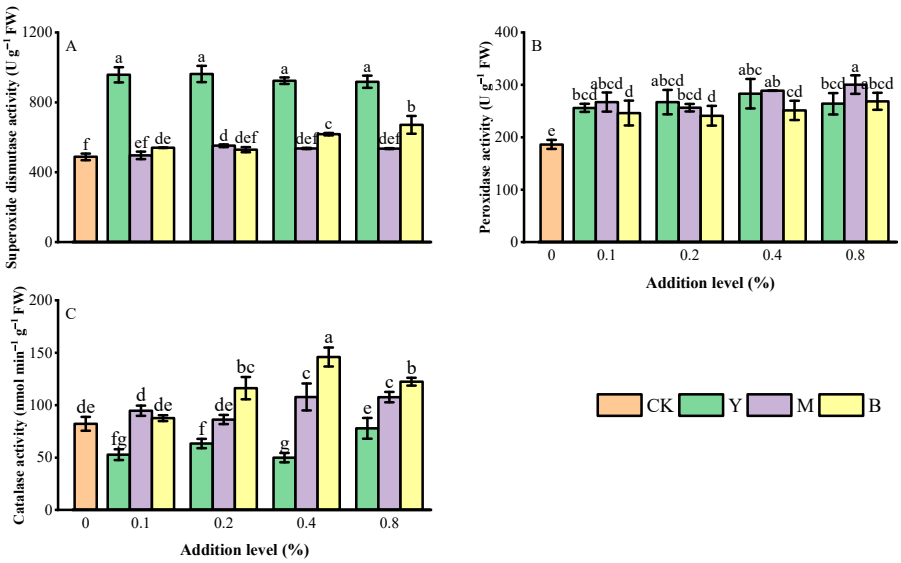

Figure 5. Effects of oil shale waste residues on the activity of superoxide dismutase (**A**), peroxidase (**B**), and catalase (**C**) in maize seedling leaves. Different lowercase letters indicate significant differences at the 0.05 probability level ($p < 0.05$), determined by one-way analysis of variance (ANOVA) and Duncan's post hoc test for significance. The vertical bar chart represents the mean ± standard deviation (SD) calculated from three repetitions. Y: fine concentrate ore; M: fine ore; B: semi-coke.

The impact of the three oil shale wast residues on catalase (CAT) activity varied depending on the type of waste residue. Specifically, both M and B treatments increased CAT activity compared to CK, with increases ranging from 4.98% to 30.95% and 6.48% to 77.42%, respectively. The maximum activity was achieved at the 0.4% addition level. In contrast, Y treatments decreased CAT activity compared to CK, with reductions ranging from 5.30% to 39.28%.

3.6. Comprehensive Evaluation

Single- and two-factor analyses of variance were conducted for the 16 indicators of maize seedlings (Table 3). The results revealed significant effects of oil shale type on growth indicators (underground fresh weight and dry weight, total fresh weight and dry weight, seedling height) and physiological–biochemical indicators (root vitality, superoxide dismutase, peroxidase, and catalase) ($p < 0.05$). The addition level also showed a significant impact on physiological–biochemical indicators (root vitality, proline, superoxide dismutase, peroxidase, catalase, and malondialdehyde) ($p < 0.05$), and there was a significant interaction between type and addition level for physiological–biochemical indicators (root vitality, proline, superoxide dismutase, catalase, and malondialdehyde) ($p < 0.05$).

Table 3. Single- and two-factor analysis of variance.

Indicators		Type		Addition Level		Type × Addition Level	
		F	*p*	F	*p*	F	*p*
X1	Height	6.436	**	0.89	ns	0.126	ns
X2	Stem width	1.566	ns	1.931	ns	0.652	ns
X3	Underground fresh weight	1.913	ns	1.849	ns	1.518	ns
X4	Underground dry weight	2.212	ns	1.796	ns	0.526	ns
X5	Aboveground fresh weight	6.557	**	1.276	ns	0.805	ns
X6	Aboveground dry weight	6.945	**	0.885	ns	0.796	ns
X7	Total fresh weight	7.146	**	1.52	ns	0.786	ns
X8	Total dry weight	7.21	**	1.165	ns	0.786	ns
X9	Root/Shoot ratio	1.067	ns	2.012	ns	0.31	ns
X10	Seedling vigor index	2.273	ns	1.583	ns	0.626	ns
X11	Root vitality	13.48	***	14.508	***	22.34	***
X12	Proline	0.639	ns	81.593	***	134.448	***
X13	Superoxide dismutase	820.503	***	4.205	*	9.575	***
X14	Peroxidase	6.738	**	4.015	*	1.186	ns
X15	Catalase	201.306	***	23.447	***	13.511	***
X16	Malondialdehyde	1.395	ns	12.374	***	5.326	**

Note: *, **, and *** indicate statistically significant difference at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, and 'ns' indicates no statistically significant difference.

Through clustering heatmap analysis of 16 indicators in maize seedlings subjected to different treatments (Figure 6), the research results reveal similarities among different indicators and demonstrate a feature of coordinated regulation. A principal component biplot was drawn for physiological and biochemical indicators and treatments (Figure 7), indicating a certain connection among indicators X11 to X16. Therefore, these findings suggest overlap and intertwining, revealing that individual indicator parameters have different effects on alleviating salt stress in maize seedlings. Thus, principal component analysis was employed to reanalyze the 16 indicators.

Using SPSS 26.0 software for the 16 indicators of maize seedlings, the Kaiser–Meyer–Olkin (KMO) value for validity testing was 0.850. The first four composite indicator contribution rates were 43.707%, 19.009%, 9.603%, and 8.499%, with a cumulative contribution rate of 80.818% (Table 4). Eigenvalues greater than 1 were selected, and the first four principal components were chosen as the main factors for the comprehensive evaluation of salt stress relief. The criterion was that the eigenvalues of these principal components were >1, and the cumulative variance contribution rate was >80%. The analysis of characteristic

vectors for different composite indicators showed that in Principal Component 1 (Table 5), total dry weight (X8), total fresh weight (X7), aboveground fresh weight (X5), aboveground dry weight (X6), seedling vigor index (X10), and underground dry weight (X4) had relatively high contribution rates, namely 0.969, 0.943, 0.927, 0.9, 0.854, and 0.83, respectively. In Principal Component 2, malondialdehyde (X16) and stem width (X2) had relatively high contribution rates, namely 0.828 and 0.777. In Principal Component 3, root vitality (X11), and superoxide dismutase (X13) had relatively high contribution rates, namely 0.704 and 0.682. In Principal Component 4, peroxidase (X14) had a relatively high contribution rate of 0.886.

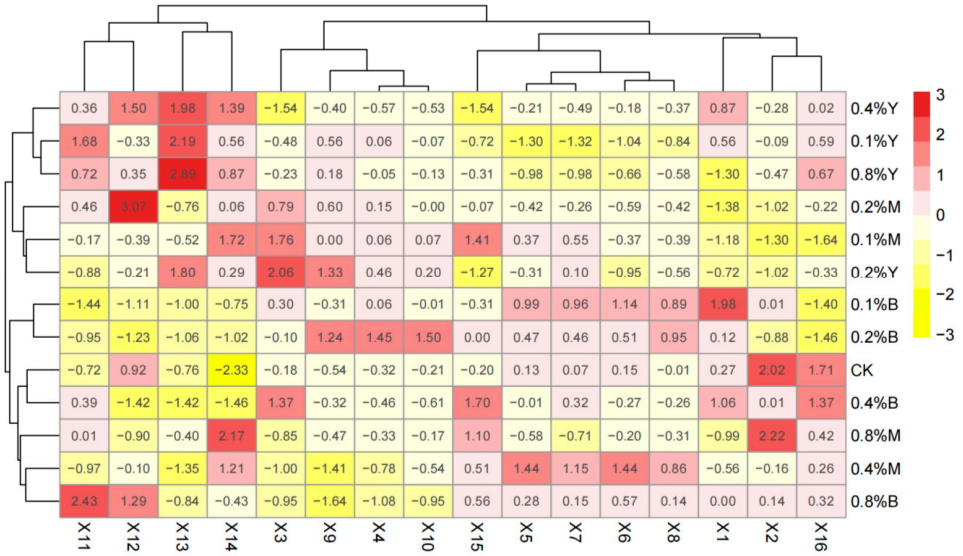

Figure 6. Clustering heat map of each indicator and treatment. X1: height, X2: stem width, X3: underground fresh weight, X4: underground dry weight, X5: aboveground fresh weight, X6: aboveground dry weight, X7: total fresh weight, X8: total dry weight, X9: root/shoot ratio, X10: seedling vigor index. X11: root vitality, X12: proline, X13: superoxide dismutase, X14: peroxidase, X15: catalase, X16: malondialdehyde. Y: fine concentrate ore, M: fine ore, B: semi-coke. 0.1%, 0.2%, 0.4%, and 0.8%, respectively, represent volume fraction additions of 0.1%, 0.2%, 0.4%, and 0.8% in the cultivation substrate.

Table 4. Principal component analysis eigenvalues and contribution rates.

Components	Initial Eigenvalues			Total Extraction Sums of Squares Loadings		
	Total	Variance Percentage	Accumulated %	Total	Variance Percentage	Accumulated %
1	6.993	43.707	43.707	6.993	43.707	43.707
2	3.041	19.009	62.716	3.041	19.009	62.716
3	1.536	9.603	72.319	1.536	9.603	72.319
4	1.36	8.499	80.818	1.36	8.499	80.818
5	0.94	5.873	86.691			
6	0.883	5.518	92.209			
7	0.595	3.719	95.928			
8	0.423	2.647	98.575			
9	0.126	0.787	99.362			
10	0.075	0.469	99.832			
11	0.019	0.118	99.949			
12	0.008	0.051	100			
13	1.21×10^{-16}	7.57×10^{-16}	100			
14	-8.16×10^{-17}	-5.10×10^{-16}	100			
15	-3.56×10^{-16}	-2.23×10^{-15}	100			
16	-7.47×10^{-16}	-4.67×10^{-15}	100			

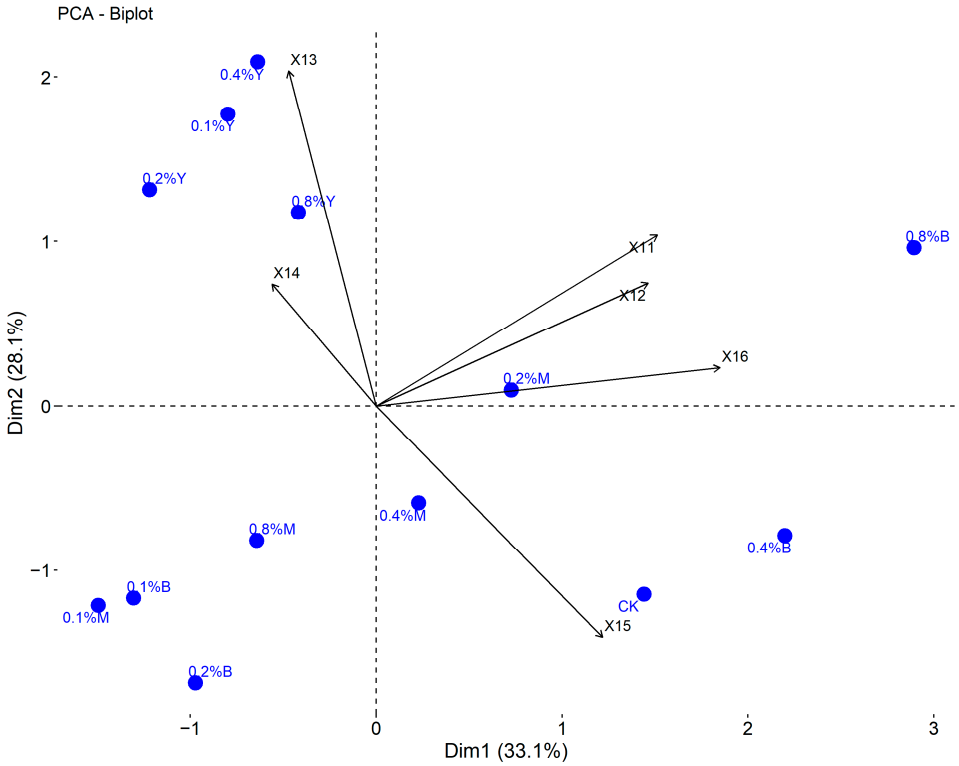

Figure 7. Dual-axis plot of physiological and biochemical indicators and treatment. X11: root vitality, X12: proline, X13: superoxide dismutase, X14: peroxidase, X15: catalase, X16: malondialdehyde. Y: fine concentrate ore, M: fine ore, B: semi-coke. 0.1%, 0.2%, 0.4%, and 0.8%, respectively, represent volume fraction additions of 0.1%, 0.2%, 0.4%, and 0.8% in the cultivation substrate.

Table 5. Loading matrix and eigenvectors for each indicator in principal components.

Variables	Loading Matrix				Eigenvectors			
	Principal Components 1	Principal Components 2	Principal Components 3	Principal Components 4	Principal Components 1	Principal Components 2	Principal Components 3	Principal Components 4
X1	0.667	0.203	0.282	−0.148	0.252	0.116	0.228	−0.127
X2	0.197	0.777	−0.252	−0.270	0.074	0.446	−0.203	−0.232
X3	0.592	−0.200	0.216	−0.178	0.224	−0.115	0.174	−0.153
X4	0.830	−0.468	0.149	−0.095	0.314	−0.268	0.120	−0.081
X5	0.927	0.180	−0.069	0.167	0.351	0.103	−0.056	0.143
X6	0.900	0.263	−0.082	0.203	0.340	0.151	−0.066	0.174
X7	0.943	0.126	−0.024	0.119	0.357	0.072	−0.019	0.102
X8	0.969	0.037	−0.010	0.121	0.366	0.021	−0.008	0.104
X9	0.635	−0.633	0.307	−0.183	0.240	−0.363	0.248	−0.157
X10	0.854	−0.410	0.089	−0.065	0.323	−0.235	0.072	−0.056
X11	−0.061	0.441	0.704	0.363	−0.023	0.253	0.568	0.311
X12	−0.047	0.554	0.392	0.009	−0.018	0.318	0.316	0.008
X13	−0.523	−0.302	0.682	−0.010	−0.198	−0.173	0.550	−0.009
X14	−0.285	−0.150	−0.017	0.886	−0.108	−0.086	−0.014	0.760
X15	0.693	0.386	−0.111	0.302	0.262	0.221	−0.090	0.259
X16	−0.005	0.828	0.288	−0.286	−0.002	0.475	0.232	−0.245

Note: X1: height, X2: stem width, X3: underground fresh weight, X4: underground dry weight, X5: aboveground fresh weight, X6: aboveground dry weight, X7: total fresh weight, X8: total dry weight, X9: root/shoot ratio, X10: seedling vigor index. X11: root vitality, X12: proline, X13: superoxide dismutase, X14: peroxidase, X15: catalase, X16: malondialdehyde.

The first principal component is:

$$F1 = 0.252X1 + 0.074X2 + 0.224X3 + 0.314X4 + 0.351X5 + 0.340X6 + 0.357X7 + 0.366X8 + 0.240X9 + 0.323X10 - 0.023X11 - 0.018X12 - 0.198X13 - 0.108X140.262X15 - 0.002X16$$

The second principal component is:

$$F2 = 0.116X1 + 0.446X2 - 0.115X3 - 0.268X4 + 0.103X5 + 0.151X6 + 0.072X7 + 0.021X8 - 0.363X9 - 0.235X10 + 0.253X11 + 0.318X12 - 0.173X13 - 0.086X14 + 0.221X15 + 0.475X16$$

The third principal component is:

$$F3 = 0.228X1 - 0.203X2 + 0.174X3 + 0.120X4 - 0.056X5 - 0.066X6 - 0.019X7 - 0.008X8 + 0.248X9 + 0.072X10 + 0.568X11 + 0.316X12 + 0.550X13 - 0.014X14 - 0.090X15 + 0.232X16$$

The fourth principal component is:

$$F4 = -0.127X1 - 0.232X2 - 0.153X3 - 0.081X4 + 0.143X5 + 0.174X6 + 0.102X7 + 0.104X8 - 0.157X9 - 0.056X10 + 0.311X11 + 0.008X12 - 0.009X13 + 0.760X14 + 0.259X15 - 0.245X16$$

According to the variance contribution analysis, the first principal component accounts for 43.707%, the second principal component accounts for 19.009%, the third principal component accounts for 9.603%, and the fourth principal component accounts for 8.449%. Combining the principal component coefficients and their corresponding variance contribution rates, a comprehensive evaluation formula is established as F = 43.707F1 + 19.009F2 + 9.603F3 + 8.449F4. Through the evaluation formula, comprehensive scores for the three types of oil shale waste residues and their four addition levels on salt-affected soil maize seedlings can be obtained (Table 6). Based on the comprehensive scores, the alleviation effects are ranked as B2 > B3 > B4 > M3 > B1 > CK. This implies that semi-coke at addition levels of 0.1% to 0.4% and 0.4% fine ore treatment exhibit a mitigating effect on salt stress, while the other treatments do not show a significant alleviation effect.

Table 6. Principal component composite scores.

Treatment	F1	F2	F3	F4	F	Rank
CK	−0.002	2.599	−1.006	−3.068	13.589	6
0.1%Y	−4.033	−1.147	1.193	−0.611	−191.796	13
0.1%M	−1.544	−1.449	−1.685	0.645	−105.749	12
0.1%B	1.898	−0.345	−1.154	−0.298	62.808	5
0.2%Y	−0.397	−2.179	1.156	−0.657	−53.252	8
0.2%M	−0.788	−0.163	0.315	0.002	−34.479	7
0.2%B	6.025	−2.887	0.058	−0.217	207.149	1
0.4%Y	−2.359	−0.045	0.807	0.531	−91.686	10
0.4%M	1.210	1.146	−1.106	1.502	76.794	4
0.4%B	2.707	1.654	1.135	−0.227	158.741	2
0.8%Y	−2.204	−0.683	0.541	0.014	−103.986	11
0.8%M	−1.879	0.639	−1.985	0.935	−81.094	9
0.8%B	1.365	2.859	1.730	1.449	142.961	3

Note—Y: fine concentrate ore, M: fine ore, B: semi-coke. 0.1%, 0.2%, 0.4%, and 0.8%, respectively, represent volume fraction additions of 0.1%, 0.2%, 0.4%, and 0.8% in the cultivation substrate.

4. Discussion

Salt stress negatively impacts various growth indicators of maize seedlings, including seedling height, leaf number, leaf area, and the fresh and dry weights of both aboveground and underground parts [9,25–27]. Similar salt-induced damage has been reported in other crops such as tomatoes [10], rapeseed [28], wheat [29], cotton [11], and clover [30]. Our research results indicate that the influence of oil shale waste residues on various growth indicators of maize seedlings is affected by the type and concentration of the waste residues. Specifically, the application of oil shale waste residues exhibits significant differences, particularly in biomass accumulation (Tables 2 and 3). Among the three types of oil shale waste residues, the semi-coke treatment increased the plant height and

biomass of maize seedlings, especially at the 0.2% semi-coke addition level, where the effect was most pronounced. Additionally, the root/shoot ratio and seedling vigor index also increased. Consistent with the results of Kul et al. [10]'s principal component analysis, growth indicators are located on the left side of the quadrant, indicating that biochar treatment, beneficial for alleviating salt stress, also falls on the left side, while the salt-treated control is located in the lower-right quadrant. Similarly, our semi-coke treatment and growth indicators are both on the left side of the quadrant. Therefore, the semi-coke treatment is beneficial for alleviating salt stress. This is attributed to the organic-rich content of semi-coke, which has higher nutrient levels than other additives, sustaining nutrient supply. This conclusion aligns with Guan et al. [31]'s findings in their study on the use of low-grade oil shale for wind-blown soil improvement, where the impact on oat growth was consistent.

The root system is the earliest part of plants to perceive salt stress, and root vitality is a crucial indicator for assessing the health of roots. Previous research has indicated that salt stress significantly reduces the root vitality of plant seedlings, such as in rice [32], rapeseed [28], and clover [30]. Additionally, the application of biochar improves the growth environment for roots, enhances soil porosity, reduces bulk density, and positively influences the morphological development of plant roots, including root length, surface area, density, and ultrastructure [33,34]. The application of biochar significantly enhances root vitality, alleviating nutrient and water uptake deficiencies in plants. These studies emphasize root improvement rather than simply increasing biomass accumulation [34,35]. Similar to biochar, oil shale waste residues exhibit similar physical and chemical characteristics, featuring a porous structure and high specific surface area [13]. Past research findings support our perspective that the three types of oil shale waste residue treatments contribute to enhancing the root vitality of maize seedlings. Salt stress lowers soil osmotic pressure, even below the 100 MPa of plant cells [8]. In such conditions, plants tend to undergo an increase in osmoprotectants, such as proline, to reduce cell water potential and enhance the absorption capacity of soil moisture [35]. Previous studies have indicated that salt stress increases the proline content in maize seedlings [9,36–38]. However, the addition of the three types of oil shale waste residues seems to alleviate salt stress, subsequently reducing the proline content in maize seedlings. We speculate that semi-coke, by increasing soil water retention capacity and sodium adsorption capability, contributes to stress mitigation [13]. This effect is similar to the action of biochar, which reduces osmotic stress by increasing soil water content and releasing mineral nutrients, thereby lowering the proline content in plant tissues [39,40].

Salt stress disrupts physiological processes in plants, particularly triggering oxidative damage. The accumulation of reactive oxygen species (ROS) is a primary cause of reduced crop productivity, adversely affecting various cellular functions such as nucleic acids, proteins, and lipids. Excessive ROS damages cell membranes, increases electrolyte leakage, and reduces membrane stability, leading to the accumulation of membrane lipid peroxidation products, such as malondialdehyde (malondialdehyde) [10,11,36,37,41]. Previous studies have reported an increase in malondialdehyde content in maize seedlings under salt stress [9,37,38]. One study, for instance, indicated that when the salt (NaCl) concentration was 100 mM, the malondialdehyde content in maize seedlings increased by 310.9% compared to the control [42]. Similar results have been reported in cotton [11], rapeseed [28], tomatoes [10], and wheat [29]. However, all three types of oil shale waste residue treatments reduced malondialdehyde content compared to the control, suggesting the potential alleviation of oxidative damage to cell membranes.

The enzymatic antioxidant system in plants, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), plays a crucial role in mitigating stress-induced ROS accumulation [10,11,28,29,38,43]. Superoxide dismutase (SOD) acts as the "first line of defense" in plants against oxidative damage by converting superoxide radicals into H_2O_2 and molecular oxygen, which are then transformed into water by POD and CAT [43]. Previous studies have indicated that salt stress increases SOD, POD, and CAT in maize

seedlings [42]. In this study, treatments with final ore and semi-coke had significant positive effects on SOD, POD, and CAT, while fine concentrate ore had positive effects on SOD and POD, especially SOD, but a negative effect on CAT. Maize seedlings with higher SOD, POD, and CAT activities help in the removal or reduction of ROS, providing better growth conditions [9,37]. In this study, semi-coke treatment enhanced antioxidant enzyme activity and showed higher biomass. This aligns with previous research using biochar treatment, which enhanced the activities of SOD, POD, and CAT in plants, reducing oxidative damage [29]. Therefore, the above results support the conclusion that all three types of oil shale waste residues activate antioxidant enzyme activity, alleviating oxidative damage to cell membranes caused by salt stress.

The stress resistance of plants is a complex process regulated by multiple genes, and evaluating salt tolerance based on a single indicator appears to be simplistic. In this study, the principal component synthesis evaluation method was employed, revealing that semi-coke treatment ranked higher than the control treatment. This indicates that semi-coke treatment helps improve the growth of maize seedlings in saline–alkali soil. Additionally, considering the proportion of each indicator in the principal components, our results support that a reduction in biomass is one of the main reasons for the poor growth of maize [36]. Given the effectiveness of semi-coke treatment in alleviating salt stress, three potential mechanisms are speculated: (1)semi-coke leads to an improvement in the root growth environment, (2) semi-coke has strong water retention capabilities, aiding in diluting salts and reducing osmotic pressure, and (3) semi-coke is rich in mineral nutrients beneficial for plant growth. Based on the results of the pot experiments, we recommend an application rate in the field ranging from 30 to 90 tons per hectare. However, the actual effects may vary under field conditions. Therefore, we plan to conduct field verification in further studies to determine the optimal application rate. Future research will delve into the molecular and soil characteristics influenced by biochar on maize seedlings, providing a more comprehensive understanding of the deeper benefits of biochar under saline–alkali conditions for maize seedlings.

5. Conclusions

This study suggests that among the three types of oil shale waste residue added to saline–alkali soil, semi-coke treatment may be an effective method to alleviate salt stress and mitigate salt damage to corn seedlings. When semi-coke treatment is applied to moderately saline–alkali soil, it enhances the root vitality of corn seedlings, reduces root osmotic stress, and increases the activity of leaf superoxide dismutase (8.38~37.55%), peroxidase (29.39~44.17%), and catalase (6.48~77.42%). This treatment also slows down the degree of cell membrane lipid peroxidation (0.02~26.49%), thereby improving the vigor index of corn seedlings under salt stress (17.39~182.61%), with the most optimal effect observed at a 0.2% addition rate. This research not only holds the promise of providing new insights for increasing crop yield in saline–alkali soil, but also offers an innovative approach to balancing the relationship between global food demand and soil health. By gaining a deeper understanding of the potential value of oil shale waste residue in agriculture, there is an opportunity to achieve sustainable utilization of this resource, providing a sustainable solution for future food production in saline–alkali areas.

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