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Physiological Responses and Phytoremediation Abilities of Cucumber (*Cucumis sativus* L.) under Cesium and Strontium Contaminated Soils

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Abstract: Soils contaminated with radionuclides pose a long-term radiation hazard to human health through food chain exposure and other pathways. The uptake, accumulation, and distribution of ^{133}Cs, individual ^{88}Sr, and combined ^{88}Sr + ^{133}Cs, with their physiological and biochemical responses in greenhouse-potted soil-based cucumber (*Cucumis sativus* L.), were studied. The results from the present study revealed that the uptake, accumulation, TF, and BCF ability of cucumber for ^{88}Sr + ^{133}Cs were greater than for ^{133}Cs and ^{88}Sr while the concentration was the same in the soil (10, 20, 40, 80, and 160 mg kg^{-1}). The highest ^{88}Sr + ^{133}Cs accumulation was 2128.5 µg g^{-1}dw, and the highest accumulation values of ^{133}Cs and ^{88}Sr were 1738.4 µg g^{-1}dw and 1818.2 µg g^{-1}dw (in 160 mg kg^{-1}), respectively. The lowest ^{88}Sr + ^{133}Cs, ^{133}Cs, and ^{88}Sr accumulation values were 416.37 µg g^{-1}dw, 268.90 µg g^{-1}dw, and 354.28 µg g^{-1}dw (10 mg kg^{-1}), respectively. MDA content was higher under ^{88}Sr and ^{133}Cs stress than under ^{88}Sr + ^{133}Cs stress. Chlorophyll content increased at 10 and 20 mg kg^{-1}; however, it decreased with increasing concentrations (40, 80, and 160 mg kg^{-1}). Proline content and the activities of CAT, POD, and SOD were lower under ^{133}Cs and ^{88}Sr than ^{88}Sr + ^{133}Cs stress. The ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs treatment concentrations sequentially induced some enzymes over 60 days of exposure, suggesting that this complex of antioxidant enzymes—CAT, POD, and SOD—works in combination to reduce the impact of toxicity of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs, especially in young leaves. It is concluded that cucumber reveals considerable phytoremediation capabilities due to unique growth potential in contaminated substrate and is suitable for the bioreclamation of degraded soils. The plant is especially applicable for efficient phytoextraction of ^{88}Sr + ^{133}Cs contamination.

Keywords: cucumber; ^{133}Cs; ^{88}Sr; soil pollution; phytoextraction; bio-accumulation

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1. Introduction

Soil contaminated with radionuclides due to anthropogenic activities, nuclear weapons testing, pollution of the nuclear power plants, accidents such as the Chernobyl disaster, and dumping of nuclear waste create significant challenges to the ecosystem [1,2]. The long life expectancy of radionuclides and their extensive presence instigate severe pollution of the soil, including agriculture and farmland [1,3]. Human beings are directly or indirectly

exposed to these pollutants in soil and waste streams through different ways, such as eating contaminated food crops or drinking contaminated water, which may cause serious harms to human health. In this regard, ^{133}Cs and ^{88}Sr are transferred to plants due to absorption by roots from the liquid phase of the soil [4,5]. The availability of radionuclides in soil solutions is influenced by a variety of factors, including climate, soil variables (such as organic matter, pH, CEC, and soil microbes), and plant characteristics such as taxonomy, morphology, and physiology [6–8]. Although Sr and Cs do not have known functions to perform in the plant body, being chemically similar to essential Ca and K, they are believed to enter into the plant body using Ca and K transporters in the cell membrane. Therefore, they compete with K and Ca ions, causing the significant reduction in their transport to the cytoplasm and an ultimate deficiency of these essential elements in plant [9–11] where they cause potential metabolic interference and growth and productivity loss [12]. Moreover, the ability of plants to absorb stable isotopes ^{88}Sr and ^{134}Cs from the soil is similar to their ability to absorb radioactive ^{90}Sr and ^{137}Cs; according to certain research, the transport and dispersion of ^{137}Cs and ^{90}Sr in the plant soil system can be very well simulated using ^{133}Cs and ^{88}Sr [13,14].

In recent years, the role of phytoremediation in the restoration of contaminated areas of ^{90}Sr and ^{137}Cs has become the focus of research [15–17]. Phytoextraction is considered as an alternate to existing physical and chemical reduction methods. The amount of soil to be treated has decreased by nearly 100 times, but this process takes time and leads to the absorption of radionuclide biomass [18,19]. Phytoextraction is suitable for those hyperaccumulator plants that have huge biomass, quick growth periods, and that move toxic pollutants from the soil to harvestable parts (leaf and stem) of the plants. However, the efficiency of plants for accumulating these toxic pollutants from the soil and transporting them to their aboveground parts depends upon the plant species, growth conditions, and soil types [20]. There has been a sparse amount of literature reporting the ability of horticultural crops for the phytoextraction of ^{90}Sr and ^{137}Cs from contaminated sites.

In the past, it has been reported that leafy vegetables, e.g., komatsuna (*Brassica rapa* var. perviridis) and mustard (*Brassica juncea*), which possess large root volumes and root surface areas, had a higher ^{137}Cs transfer value than the root vegetables radish (*Raphanus sativus*) and turnip (*Brassica rapa* var. glaba) [2]. Moreover, in the phytoremediation of Cs-polluted soil, oilseed rape and New Zealand spinach were found to be promising plant species, as were pumpkin and sunflower for Sr-polluted soil [1]. However, for the phytoremediation of Cs- and Sr-polluted soil, not only the absorption ability but also the characteristics of the species used should be considered and evaluated [1]. Therefore, we assumed that cucumber plants used in the present study would have high Sr and Cs absorption and transport ability owing to their rapid growth, extensive root system, and high survival and tolerance to excessive concentrations of heavy metals. Moreover, concern may arise for its end-use, and for this purpose we would like to recommend that contaminated residual biomass can be utilized for bioenergy production in addition to its safe disposal options, e.g., in a landfill [21–23]. Thus, integrating phytoremediation with valuable material and bioenergy production can make the process economically viable. Thus, in the present study, cucumber plants were utilized to assess their efficacy to tolerate combined ^{90}Sr and ^{137}Cs stress. The specific objectives of the present study were to evaluate the bioaccumulation, transportation, and distribution of individual ^{133}Cs and ^{88}Sr and combined ^{88}Sr + ^{133}Cs and to assess the physiological and biochemical responses of cucumber (*Cucumis sativus* L.) grown in greenhouse-potted soil. In addition, we analyzed the phytoextraction efficiency in the plants in order to assess whether the cucumber plants can grow and remediate the ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs in contaminated soils.

2. Materials and Methods

2.1. Soil Collection and Seedling Growth

The soil was collected from the nursery garden center of the Southwest University of Science and Technology, Mianyang, China. Firstly, collected soil samples were air dried

and then grinded and passed through a 2 mm sieve. The pH values of soil in water (H_2O) and potassium chloride (KCl) were determined using a pH meter, and soil organic matter (SOM) was determined according to the Walkley–Black method. Deionized water (5 mL) was mixed with 1 g of grinded and sieved soil, and the soil's electrical conductivity was measured. The primary physical and chemical characteristics of the soil were pH H_2O = 7.0, pH KCl = 6.22, soil organic matter percentage = 1.24, and electrical conductivity (mS/cm) = 1.12. Cucumber plant seeds were purchased from the Mianyang seed company, the nutritional soil was filled with small bags of polyethylene for seedlings, and seeds were planted approximately 1 cm deep in uncontaminated soil (one seed in each bag).

2.2. Treatment and Experimental Design

The pot experiment was carried out in the greenhouse of Southwest University of Science and Technology in Mianyang, China (E = 104°41′, N = 31°32′). The sieved soil was kept on a waterproof sheet and mixed with ^{88}Sr $(NO_3)_2$,$^{133}CsCl$, and ^{88}Sr $(NO_3)_2$ + $^{133}CsCl$ solutions to obtain appropriate concentrations in soil (Table 1). The soil was then allowed to stabilize for at least 30 days. Uncontaminated soil was used as a control. Each pot (25 cm in diameter, 20 cm in height) was filled with 4.5 kg of concentrated soils. Three uniform 15 day-old seedlings were transferred into each pot, and three pots of each treatment and three replicates were used in a randomized complete block design (RCBD). Plants were irrigated with tap water up to 100% for their field potential to maintain 41.9% soil moisture. Every pot was kept on the plastic dishes to collect leachates, which were applied periodically to the pot soil to minimize treatment loss. After transport of the seedlings, the pot trial lasted for 60 days.

Table 1. Concentration of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs applied in soils.

Treatment (mg/kg^{-1})	Treatment					
	0 (ck)	**1**	**2**	**3**	**4**	**5**
^{88}Sr	0	10	20	40	80	160
^{133}Cs	0	10	20	40	80	160
^{88}Sr + ^{133}Cs (1:1)	0	10 + 10	20 + 20	40 + 40	80 + 80	160 + 160

Note: 0 is the control, each treatment is repeated three times.

2.3. Growth Measurements

The growth of the whole plant was measured after the harvest. Stainless steel scissors were used to cut the plant specimens into two parts, namely shoot and root, after rinsing with distilled water. Specimens were first measured using a scale to determine shoot height (cm), root length (cm), and leaf area (cm^2), and after that they were air dried in a natural cool environment and well-ventilated location, and then oven-dried for 24 h at (68 °C ± 2 °C). The dry weight (g $plant^{-1}$) of each part was weighed to calculate the biomass.

2.4. Assessing the ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs Accumulation

Inductively coupled plasma-optical emission spectrometry (ICP-MS; version 715-ES ICP-MS; version, Palo Alto, CA, USA) with the acid digestion method was used to assess the plant concentrations of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs in shoot and root, as stated by [24]. In brief, 15 mL HNO_3 and $HClO_4$ (3:1 v/v) were used to acid digest 0.5 g of dried shoot tissue and 0.3 g of dry root tissue. The digestive vessels of the specimens were kept for 15 min in a microwave-assisted digestion system (MDS-6G) between 120 and 190 °C, for 15 and min at 190 °C for 30 min at 190 °C. Finally, the digested specimens were diluted with deionized water to a final volume of 50 mL for individual ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs determinations. The concentrations and accumulations of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs in the plants′ roots and shoots were calculated according to [25].

2.5. Phytoextraction Potential

The plant extraction capacity was determined by measuring the biological concentration factor (BCF) and transfer factor (TF) of the nuclide. BCF is the ratio of nuclide concentration in the aboveground part of the whole plant to nuclide concentration in the soil, and TF is the ratio of the nuclide concentration in the aboveground part of the plant to the root of the plant [26].

BCF and TF were calculated by these formulas:

$$BCF = C_{shoot}\ (\text{mg kg}^{-1}\ \text{dw}) / C_{soil}\ (\text{mg kg}^{-1}\ \text{dw})$$

$$TF = C_{shoot}\ (\text{mg kg}^{-1}\ \text{dw}) / C_{root}\ (\text{mg kg}^{-1}\text{dw})$$

where C_{shoot}, C_{root}, and C_{soil}, respectively, are concentrations of nuclides in shoot, root, and soil.

2.6. Assessing the Physiological and Biochemical Indexes

The contents of chlorophyll content, malondialdehyde (MDA), proline, superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were measured from the plant leaves. Total chlorophyll content was calculated using the Li technique and quantified at 652 nm using the 95% ethanol colorimetric method [27]. The content of MDA was determined by total bile acid colorimetry, as in [27]. The content of the proline was determined by the Bates method [28]. The activity of SOD and POD was determined by Li's method, and SOD activity was assayed based on the reduction of NBT (nitroblue tetrazolium). This reaction was conducted in sodium phosphate buffer (50 mM, pH 7.8) containing 100 µL of enzyme extract, 2 µM riboflavin, 65 µM NBT, 13 µM methionine, and 1 µM ethylenediamine tetraacetic sodium. The 3 mL reaction mixture was initialed by illumination for 2 min at 25 °C, and the absorbance of blue formazan was measured with a spectrophotometer (UV-3802, Unico, Shanghai, China) at 560 nm. One unit of SOD activity (U) was defined as the amount of enzyme that caused 50% inhibition of NBT reduction. POD activity was determined by measuring the absorbance changes at 470 nm and 25 °C. The reaction was performed in a 3 mL solution. A 10 µL volume of enzyme solution was added to 2.99 mL of sodium phosphate buffer (50 mM, pH 6.0) containing 18.2 mM guaiacol and 4.4 mM H_2O_2 as substrates. POD activity was defined as the amount of enzyme that caused an increase in absorbance at 470 nm of 0.001 per minute. CAT activity was measured by monitoring the decrease of H_2O_2 at 240 nm for 1 min at 25 °C. The 3 mL reaction mixture contained 100 µL of enzyme extract and 2.9 mL of sodium phosphate buffer (50 mM, pH 6.0) containing 10 mM H_2O_2. CAT activity was calculated as the amount of enzyme that caused a reduction in absorbance at 240 nm of 0.01 per minute [27]. The experimental results are the average of three replicates.

2.7. Statistical Analysis

The data were tested for normalities and variance homogeneity, and they were log-transformed to correct the deviations from those assumptions when necessary. Treatment means were compared using a two-way analysis of variance. All measurements were checked by Microsoft Excel 2013, IBM SPSS Statistics V22.0, and the Origin Pro 8.0 mapping software.

3. Results

3.1. Growth Characteristics of Cucumber under ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs Stress

Plant growth is a primary stress indicator, and its response to any stress can predict the level of toxicity in the environment. In this context, the measuring growth response of cucumber to ^{133}Cs, ^{88}Sr, and ^{88}Sr + ^{133}Cs was one of the primary objectives of this study. The growth in the form of shoot height, root length, and leaf area (Figure 1) was not significantly reduced by ^{88}Sr and ^{133}Cs individual treatments or ^{88}Sr + ^{133}Cs in combination. The applications of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs (at 10 mg kg^{-1}) significantly decreased shoot

height, root length, and leaf area in comparison to the control, but higher concentrations (20, 40, 80, 160 mg kg^{-1}) did not cause any further significant decrease in plant growth characteristics. ^{133}Cs and ^{88}Sr individually and in combination produced non-significant stress even at the lowest concentration of 10 mg kg^{-1} and reduced shoot length, root length, and leaf area. This suggested the degree of toxicity produced by the two nuclides. Higher shoot height, root length, and leaf area of ^{88}Sr (99.93 cm, 13.23 cm, 93.62 cm^2, respectively), ^{133}Cs (80.40 cm, 16.46 cm, 76.82 cm^2, respectively), and ^{88}Sr + ^{133}Cs (105.36 cm, 17.5 cm, 99.09 cm^2, respectively) were recorded at 10 mg kg^{-1} concentrations. However, lower shoot height, root length, and leaf area of ^{88}Sr (78.3 cm, 11.56 cm, 48.28 cm^2, respectively), ^{133}Cs (62.23 cm, 12.43 cm, 27.80 cm^2, respectively), and ^{88}Sr + ^{133}Cs (83.33 cm, 12.83 cm, 52.52 cm^2, respectively) were recorded at 160 mg kg^{-1} concentrations. The individual ^{133}Cs, at its highest concentration, showed the significantly highest shoot height, root length, and leaf area reduction compared to ^{88}Sr and ^{88}Sr + ^{133}Cs, proving itself to be more toxic.

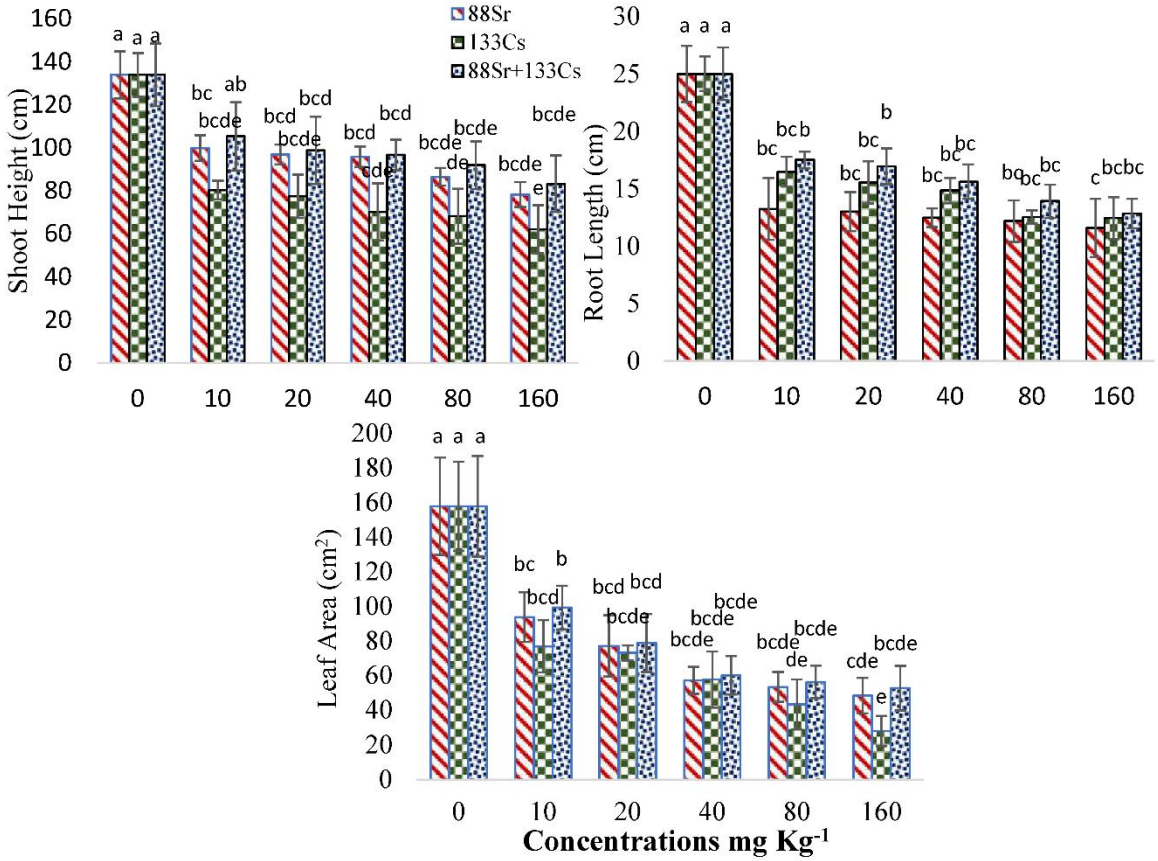

Figure 1. Shoot height, root length, and leaf area responses of cucumber to various concentrations (0 to 160 mg kg^{-1}) of ^{88}Sr, ^{133}Cs, and ^{88}Sr +^{133}Cs treatments. The columns sharing different letters are significantly different at $p < 0.05$. The error bars indicate standard deviation.

3.2. Effect of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs on the Biomass Distribution of Cucumber

The effects of ^{88}Sr and ^{133}Cs individually and ^{88}Sr + ^{133}Cs combined on the shoot and root dry biomass showed (Table 2) that the shoot and root biomass in ^{88}Sr-, ^{133}Cs-, and ^{88}Sr + ^{133}Cs-treated plants were significantly lower compared to their controls. It was observed that the ^{88}Sr + ^{133}Cs treatment had lower effects and produced higher shoot biomass of 6.297 ± 0.18 g dw $plant^{-1}$ at the 10 mg kg^{-1} level than did the ^{88}Sr + ^{133}Cs treatments, while minimum shoot biomass was recorded at the 160 mg kg^{-1} level of ^{133}Cs (2.054 ± 0.18 g dw $plant^{-1}$). The maximum root biomass (1.499 ± 0.06 g dw $plant^{-1}$) produced by ^{88}Sr + ^{133}Cs at a 10 mg kg^{-1} concentration was significantly the highest biomass value among all treatments except for the control (Table 2). It was interesting that

this treatment showed non-significant variation in root biomass up to 40 mg kg^{-1} levels and declined significantly at 80 and 160 mg kg^{-1} levels.

Table 2. Effect of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs on the biomass distribution of cucumber.

	Concentration (mg kg^{-1})	Plant Biomass (g dw $Plant^{-1}$)		
		Shoot	Root	Total
^{88}Sr	0	9.443a	2.362a	11.80a
	10	4.848bc	1.133cd	5.981bcd
	20	4.431bc	1.040de	5.472bcd
	40	4.321bc	0.995de	5.316bcd
	80	4.193bc	0.966de	5.160bcd
	160	3.501bc	0.815ef	4.317bcd
Treatment mean for ^{88}Sr		**5.122AB**	**1.218AB**	**6.341AB**
^{133}Cs	0	9.443a	2.362a	11.80a
	10	3.373bc	0.815ef	4.188bcd
	20	3.266bc	0.787ef	4.054bcd
	40	3.217bc	0.610fg	3.828cd
	80	2.838bc	0.592fg	3.431cd
	160	2.054c	0.470g	2.525d
Treatment mean for ^{88}Sr		**4.031B**	**0.939B**	**4.971B**
^{88}Sr + ^{133}Cs	0	9.443a	2.362a	11.80a
	10	6.297ab	1.499b	7.797b
	20	5.709bc	1.378bc	7.087bc
	40	5.083bc	1.209bcd	6.293bcd
	80	4.392bc	1.068de	5.460bcd
	160	3.836bc	0.925de	4.762bc
Treatment mean for ^{88}Sr ^{133}Cs		**5.793A**	**1.406A**	**7.199A**
Treatment × concentration interaction		**NS**	**NS**	**NS**

Note. Different lowercase letters, in a single column, show significant differences among concentration means at $p < 0.05$, while different upper case letters, in a single column, show significant differences among the treatment means at $p < 0.05$. NS = non-significant.

3.3. ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs Bio-Accumulation in Plant and Phytoextraction Potential

The ^{88}Sr +^{133}Cs accumulation in total plants was shown to be significantly higher than that of ^{88}Sr and ^{133}Cs among all treatment concentrations (Table 3); however, accumulation in shoot and root was significant with ^{88}Sr and ^{133}Cs at the 160 mg kg^{-1} level. Similarly, at treatment concentrations of 10, 20, and 40 mg kg^{-1}, the ^{88}Sr + ^{133}Cs accumulation in both root and shoot were non-significant; the highest accumulation in the shoot part was 1514.4 ± 240.1 µg g^{-1}dw detected in ^{88}Sr + ^{133}Cs followed by 1196.8 ± 47.78 µg g^{-1}dw in ^{88}Sr at 160 mg kg^{-1} treatment concentrations. However, the ^{88}Sr and ^{133}Cs, individually and combined, showed significant accumulation in root, namely 621.37 ± 65.5^{a} µg g^{-1} dw for ^{88}Sr, 606.16 ± 95.6 µg g^{-1} dw for ^{133}Cs, and 614.12 ± 37.3 µg g^{-1} dw for ^{88}Sr + ^{133}Cs. The highest total plant accumulation was observed (2128.5 ± 219.2 µg g^{-1}dw) at ^{88}Sr + ^{133}Cs, while the lowest total plant accumulation was observed (1738.4 ± 178.9 µg g^{-1}dw) at ^{133}Cs (160 mg kg^{-1}) treatment concentrations. This revealed that the most important factor affecting the content of ^{88}Sr + ^{133}Cs in cucumber plants was the concentration of ^{88}Sr + ^{133}Cs in the soil.

The total plant BCF of ^{88}Sr + ^{133}Cs was significantly higher than that of ^{88}Sr and Cs. However, shoot BCF was significantly the highest in ^{88}Sr + ^{133}Cs among all treatments (Table 3). The highest total plant values of BCF for ^{88}Sr (35.42), ^{133}Cs (26.89), and ^{88}Sr + ^{133}Cs (41.63) were recorded at 10 mg kg^{-1} treatment concentrations. Moreover, the ^{88}Sr + ^{133}Cs was found to have the highest TF values (2.78 and 2.51 at 80 and 160 mg kg^{-1} concentrations, respectively), while values of ^{88}Sr and ^{133}Cs were found to be significant (1.97, 1.93) at 160 mg kg^{-1} treatment concentrations.

Table 3. The effect of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs on the bio-accumulation of plants and phytoextraction potential.

Treatments	Concentrations	Accumulation (μg. g dw^{-1})			TF	BCF	BCF
		Shoot	Root	Total Plant	Shoot	Shoot	Total Plant
^{88}Sr	0	-	-	-	-	-	-
	10	156.23fg	198.05f	354.28	0.79e	15.62bcd	35.42ab
	20	226.95fg	341.52cde	568.46de	0.67de	11.34cd	28.42bc
	40	448.64efg	457.91abc	906.55cd	0.98d	11.21cd	22.66bcd
	80	962.44bcd	579.07ab	1541.51bc	1.65c	12.03bcd	19.26cde
	160	1196.84abc	621.38a	1818.22	1.97b	7.48d	11.36ed
Treatment mean for ^{88}Sr		**598.22B**	**439.58A**	**1037.80AB**	**1.212B**	**11.53**	**23.42AB**
^{133}Cs	0	-	-	-	-	-	-
	10	125.79g	143.12f	268.90f	0.88e	12.57bcd	26.89bcd
	20	204.27fg	217.48ef	421.74ef	0.94d	10.21cd	21.08bcd
	40	449.02efg	240.05def	689.06d	1.87c	11.22cd	17.22cde
	80	838.6cde	426.96abc	1265.5c	1.97b	10.48cd	15.81def
	160	1132.27abc	606.16a	1738.4b	1.93b	7.07d	10.86ef
Treatment mean for ^{133}Cs		**549.99B**	**326.75B**	**876.72B**	**1.518B**		**18.37B**
^{88}Sr + ^{133}Cs	0	-	-	-	-	-	-
	10	257.65fg	158.72f	416.37ef	1.61c	25.76a	41.63a
	20	422.69efg	241.39def	664.08de	1.73b	21.13ab	33.20ab
	40	648.1def	401.38bcd	1049.4cd	1.59c	16.20bcd	26.23cde
	80	1365.01ab	488.59abc	1853.5ab	2.78a	17.06bc	23.16cde
	160	1514.45a	614.13a	2128.5a	2.51ab	9.46cd	13.30ef
Treatment mean for ^{88}Sr + ^{133}Cs		**841.58A**	**380.84AB**	**1222.37A**	**2.044A**	**22.45A**	**27.505A**
Treatment × concentration interaction		**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

Note. Different lowercase letters, in a single column, show significant differences among concentration means at $p < 0.05$, while different upper case letters, in a single column, show significant differences among the treatment means at $p < 0.05$. NS = non-significant.

3.4. The Effect of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs Extraction Efficiency on Cucumber

The extraction productivity of cucumber in ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs was determined, and the findings are summarized in Table 4. The results showed that the concentrations of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs in the soil; plant attentiveness; and the amount of plants and pots in the soil steadily rose as the concentration in the soil increased. However, the total plant biomass and the ratio of plant/content in the pot (%) decreased gradually. In the 10 mg kg^{-1} treatments, the contents of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs were highest among plants with contents in pots, which was 7.06, 3.74, and 10.80% for ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs, respectively. In the 160 mg kg^{-1} treatment, the ratio was lowest, i.e., 1.63%, 0.91%, and 2.11% for ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs, respectively.

Table 4. The effect of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs extraction efficiency of cucumber.

^{88}Sr Treatment					
Concentration in Soil (mg/kg)	Concentration in Plant (mg kg^{-1} DW)	Biomass of Total Plant (g /Plant)	Contents in Plant (mg/Plant)	Contents in Pot (mg/Pot)	Ratio b/w Contents in the plant\Contents in a Pot (%)
0	-	11.80	-	-	
10	177.14	5.98	1.059	45	7.06
20	284.23	5.47	1.554	90	5.18
40	453.27	5.31	2.406	180	4.01
80	770.75	5.16	3.977	360	3.31

Table 4. *Cont.*

^{88}Sr Treatment					
Concentration in Soil (mg/kg)	**Concentration in Plant (mg kg^{-1} DW)**	**Biomass of Total Plant (g /Plant)**	**Contents in Plant (mg/Plant)**	**Contents in Pot (mg/Pot)**	**Ratio b/w Contents in the plant\Contents in a Pot (%)**
160	909.10	4.31	3.918	720	1.63
^{133}Cs treatment					
0	-	11.80	-	-	
10	134.45	4.18	0.562001	45	3.74
20	210.87	4.05	0.854024	90	2.84
40	344.53	3.82	1.316105	180	2.19
80	632.77	3.43	2.170401	360	1.80
160	869.21	2.52	2.190409	720	0.91
^{88}Sr + ^{133}Cs treatment					
0	-	11.80	-	-	
10	208.18	7.79	1.621	45	10.80
20	332.04	7.08	2.350	90	7.83
40	524.74	6.29	3.300	180	5.5
80	926.79	5.46	5.060	360	4.21
160	1064.2	4.76	5.065	720	2.11

3.5. Physiological and Biochemical Response of Cucumber to ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs Stress

The chlorophyll, MDA, and proline contents of cucumber are shown in Figure 2. The results reveal that the chlorophyll content of ^{88}Sr + ^{133}Cs was observed to be high at each concentration as compared to ^{88}Sr and ^{133}Cs. There were non-significant differences detected at 10, 40, and 80 mg kg^{-1} concentrations of ^{88}Sr and ^{133}Cs. The chlorophyll content was observed to be high at low concentrations; however, it decreased as the concentration of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs increased in the soil. The chlorophyll content was detected to be higher in the control (80.30) followed by 70.84, 64.97, and 75.34 mg g^{-1}FW at 10 mg kg^{-1} of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs, respectively. Similarly, the lowest chlorophyll content was detected (35.25, 34.23 mg g^{-1}FW) at (160 mg kg^{-1}) of ^{88}Sr and ^{133}Cs, respectively. It was also observed, as seen in Figure 2, that the MDA content of the cucumber plant was increased by increasing the ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs concentrations in soil. The maximum MDA content was detected for ^{88}Sr at all concentrations; compared with ^{133}Cs and ^{88}Sr + ^{133}Cs, the highest MDA content (0.333 µmol g^{-1}FW, 0.301 µmol g^{-1}FW, and 0.256 µmol g^{-1}FW) were observed at 160 mg kg^{-1} treatment concentrations of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs, respectively. Similarly, the lowest MDA were observed at 10 mg kg^{-1} of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs. The MDA content of the cucumber plant was increased at different concentrations of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs compared with the control. Figure 2 demonstrate that the proline content of cucumber leaves under ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs stress was increased as the concentration increased in the soil. Similarly, variable proline contents were detected in the ^{88}Sr and ^{88}Sr + ^{133}Cs at concentrations of 10, 40, and 80 mg kg^{-1} compared to ^{133}Cs. The highest proline content of 39.81 µg g^{-1}FW ^{88}Sr was observed at the 40 mg kg^{-1} treatment, and 39.54 µg g^{-1}FW and 41.10 µg g^{-1}FW were detected with treatment of 160 mg kg^{-1} of ^{133}Cs and ^{88}Sr + ^{133}Cs, respectively, with significant values ($p < 0.001$). Under the stress of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs, the rapid accumulation of proline was observed in cucumber as compared to their controls.

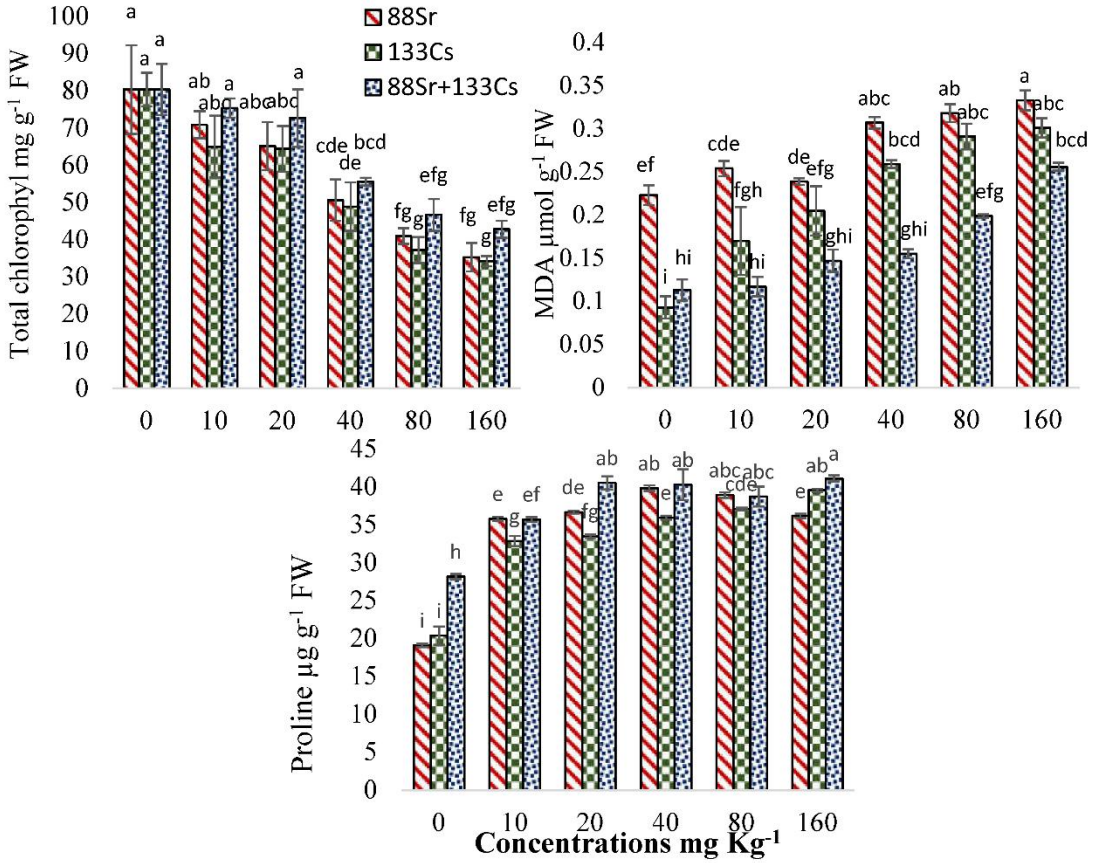

Figure 2. The effect of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs stress on the chlorophyll, MDA, and proline content of cucumber to various concentrations (0 to 160 mg kg^{-1}) of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs treatments. The columns sharing different letters are significantly different at $p < 0.05$.

The effect of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs stress on the CAT, POD, and SOD activity of cucumber plants are presented in Figure 3. The results demonstrated that the CAT activity increased with increases in the ^{88}Sr and ^{88}Sr + ^{133}Cs concentrations in soil. The highest CAT activities were 72.47 µg min^{-1}), 62.64 µg min^{-1}, and 102.49 µg min^{-1} detected at 160 mg kg^{-1} concentration of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs treatments, respectively, with significant values ($p < 0.001$). The activity of CAT in the ^{88}Sr + ^{133}Cs treatment was higher than the activity of CAT in the treatments of ^{88}Sr and ^{133}Cs. It could be concluded from the results that ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs had positive influences on the activity of CAT at high concentrations. The results from Figure 3 demonstrated that the POD activity was increased as ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs treatment concentrations increased. The maximum POD activity was observed (109.78 µg min^{-1}) for the ^{88}Sr + ^{133}Cs treatment at 160 mg kg^{-1} concentration. However, the lower POD activity was observed at 10 mg kg^{-1} of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs treatments. The results of SOD activity significantly increased as the concentration of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs increased compared with the control. The highest SOD activities (136.79 µg min^{-1}, 93.66 µg min^{-1}, and 158.49 µg min^{-1}) were recorded at the 160 mg kg^{-1} concentration of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs treatments, while, the lowest SOD activity was 58.40 µg min^{-1}, 45.90 µg min^{-1}, and 114.45 µg min^{-1} for ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs, respectively.

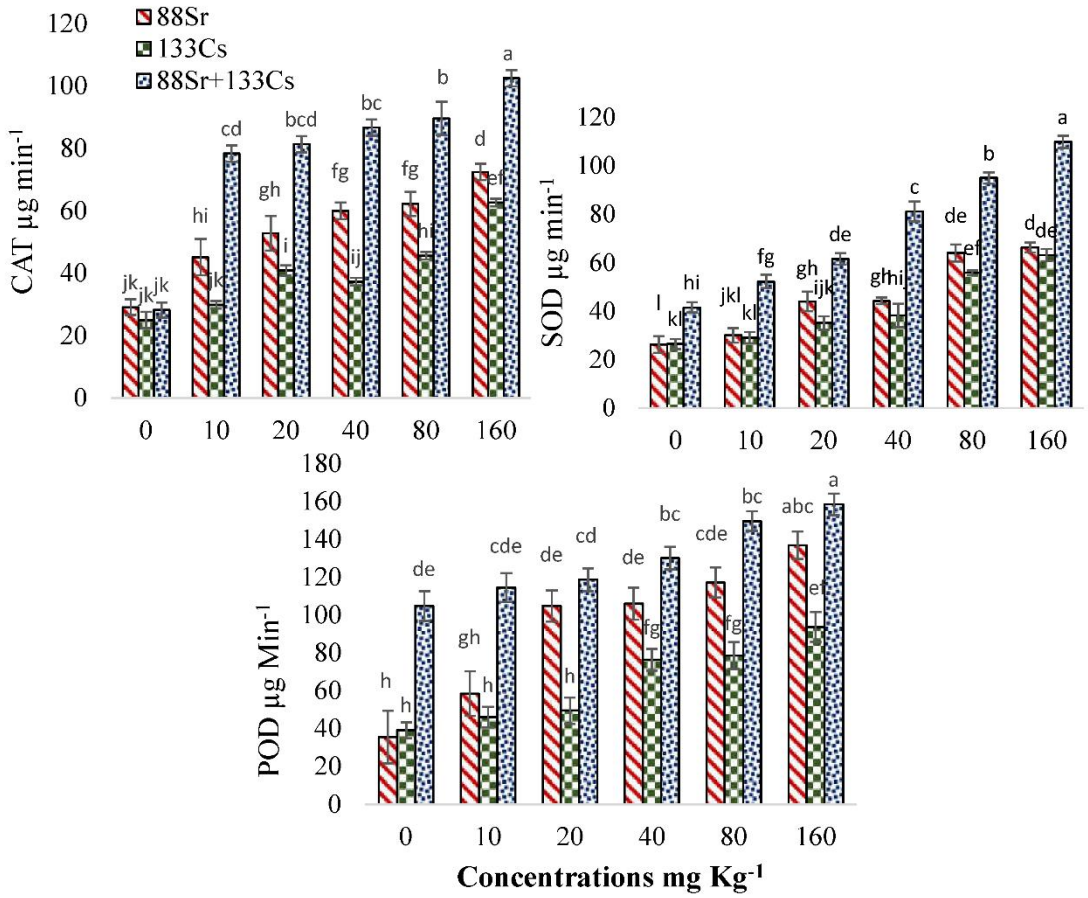

Figure 3. The effect of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs stress on the activity of antioxidant enzymes of CAT, POD, and SOD of cucumber to various concentrations (0 to 160 mg kg^{-1}) of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs treatments. The columns sharing different letters are significantly different at $p < 0.05$. The error bars indicate standard deviation.

4. Discussion

4.1. Growth Characteristics of Cucumber under ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs Stress

The role of ^{88}Sr and ^{133}Cs in plant nutrition is unclear. According to several scientists, low concentrations of heavy metal ions in the soil solution can enhance plant growth, while a high concentration of metals such as cesium and chromium in the soil can be toxic to plants and restrict plant growth [29,30]. Plants have different abilities to absorb, transfer, and isolate radionuclides, and it is a well-known fact that Cs does not have any important role in plant growth and metabolism, and hence its higher accumulation in plant biomass may create toxic effects. ^{133}Cs also reduced root length at lower and higher concentrations as compared to ^{88}Sr alone and ^{88}Sr + ^{133}Cs. Our findings confirm the observations of [5], who found the same growth effects of Cs on *Plantago major*. The reduction in growth is a result of physiological and metabolic impairment caused by metal stress. Since metal stress triggers oxidative stress in plant cells [31], which is obvious from the hyper-regulation of anti-oxidative defense genes against Cs stress [32], a reduction in growth could therefore be the result of such metabolism. Another possible cause of growth reduction can be traced to Cs competitiveness with K. The authors in [33] concluded that Cs uses K channels during its uptake, which causes K deficiencies in the cells. The authors in [32] also showed that Cs enhances oxidative stress when applied in a K-deficient medium. Due to such metabolic and physiologically adverse effects, Cs causes significant losses to growth in many sensitive plants.

Different concentrations of ^{88}Sr, when applied individually, also significantly reduced cucumber growth (Figure 1), but as described earlier, its effects were less than those of ^{133}Cs. Like Cs, Sr also does not have important functions in the plant body. Therefore, Sr has also been reported as a toxic heavy metal with its stable isotopes. The Sr toxicity effects on growth from hydroponic experiments have been reported in other plants such as *Oryza sativa* [34], *Brassica juncea* [35], *Arabidopsis thaliana* [36], *Solanum tuberosum* [37], and *Plantago major* [5]. Such findings confirm the toxic effects of ^{88}Sr on plant growth. The results of ^{88}Sr + ^{133}Cs combined application, at all concentrations, were statistically similar with Sr, although it produced the lowest reduction in shoot length, root length, and leaf area. This suggests that both nuclides when combined masked much of the toxic effects of each other.

4.2. Effect of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs on the Biomass Distribution of Cucumber

Since shoot and root biomass are directly proportional to each other, shoot length and root length results thus coincide with them and prove ^{133}Cs to be more toxic than all other treatments, particularly at higher concentration levels. It is reported that heavy metal ions, even at low concentrations in a soil solution, may decrease plant growth, while their excessive or higher concentrations can be toxic to plants and therefore limit plant growth dangerously [38,39]. This pattern shows that the nuclides when combined can reduce the toxic effects on the growth of plants. Although the mechanism behind this is yet not understood, but can nevertheless open new insights of research, it is documented [40] that plants show cross-protection, a reaction against stress in which one stress may protect the plant against others. ^{133}Cs alone showed significantly high deleterious effects on root biomass from its lowest to highest concentrations compared to all other treatments. However, its effects were non-significant from 10 to 40 mg kg^{-1} applied concentrations in soil. The ^{88}Sr showed non-significant effects to those of ^{133}Cs on root biomass at 10 and 20 mg kg^{-1}, while it produced significantly higher root biomass at concentrations of 20 mg kg^{-1} an onward than did ^{133}Cs. The findings of [5,34,41] suggest that root growth was not decreased in *Triticum aestivum*, *Glycine max,* and *Plantago major* at low concentrations; however, our results vary from these findings and suggest that even at lowest concentrations (10 mg kg^{-1}), the reduction effects were significant. The authors in [42], while studying the effects on *Amaranthus mangostanus*, found that lower concentrations of ^{133}Cs and ^{88}Sr did not affect the above ground and below ground biomass. They also found that ^{88}Sr was a more hazardous to reduce crop biomass than ^{133}Cs. These results are antagonistic to our findings. The effects of ^{88}Sr and ^{88}Sr + ^{133}Cs treatments were statistically similar on the shoot and root biomass of cucumber as compared to ^{133}Cs; statistically, it was shown that ^{88}Sr + ^{133}Cs treatment had a lower impact on the biomass of cucumber than did ^{88}Sr and ^{133}Cs alone. This suggests that ^{88}Sr, when applied in combination with ^{133}Cs, has some mitigation properties to the adverse effects of ^{133}Cs, which resembles a phenomenon of cross-protection in plants.

4.3. Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs Bio-Accumulation in Plant and Phytoextraction Potential

It can be seen from Table 3 that a slight increase in ^{88}Sr + ^{133}Cs concentration in the soil does not affect its bioaccumulation in the root and shoot of cucumber. Similar results have been reported in *Arabidopsis thaliana* [32], *Pennisetum purpureum* (up to 3 mM; [43]), *Calla palustris* (0.5 and 1 mM; [44]), and *Ocimum basilicum* (up to 0.4 mM; [33]). Earlier findings suggest that the uptake and distribution of nuclides in plants are dependent on nutrient metabolism, especially K and Ca [29]. The lower accumulation of ^{133}Cs can be attributed to the levels of K uptake and regulation. The authors in [5] suggested that K uptake was not affected by low concentrations of Cs in the external medium in *Plantago major*. However, increasing concentrations decreased the uptake of K significantly [5], which may influence the higher accumulation of Cs in shoots and roots. The bioaccumulation of nuclides in the root at the highest (160 mg_ concentration was non-significant among all treatments, suggesting statistically similar effects. The increased bioaccumulation of ^{88}Sr has been suggested in many previous studies [5,32,45,46], but they suggested its hyper-accumulation

over ^{133}Cs. In contrast, our results suggest that bioaccumulation of ^{88}Sr + ^{133}Cs was higher in shoot, while in root the ^{88}Sr accumulation was higher than the ^{133}Cs. Since the results of ^{88}Sr + ^{133}Cs were non-significant to those of ^{88}Sr in root, it can therefore be concluded that both produced similar results in their root bioaccumulation (Table 2). The authors in [45] suggested that total (in the whole plant) bioaccumulation of Cs is higher than that of Sr in *Amaranthus mangostanus*. They also concluded that *A. mangostanus* has more Cs absorption and accumulation ability. This suggests that there are some selective mechanisms in different plants for the uptake and accumulation of Sr and Cs.

The values of BCF > 1 indicate that the plant is an accumulator, while the values of BCF > 10 indicate that the plant has the potential to be a hyper-accumulator, while the value of TF of each nuclide is used to evaluate the capacity of a plant to transfer a nuclide from the root to the shoot. This value is defined as the ratio of the metal concentration in the shoot to that in the root of plants. The value of TF > 1 indicates that the plant is effective in the translocation of metal from its root to shoot. The TF values increased with an increase in concentration levels in all treatments [47,48]. Notably, BCF and TF of ^{88}Sr and ^{133}Cs found in our study were high compared to those reported for other species such as *Amaranthus mangostanus* L. [45], *Raphanus sativus* L. [42], and *Gypsophila paniculata* [49]. Table 4 indicates that when the intensity of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs in the soil was high, it was more difficult to uptake^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs from the soil, and it would take additional time to apply cucumber to remediate the high concentrations of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs from contaminated soil. In this test, when the concentration in the soil of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs was 10 mg kg^{-1}, it needed to grow cucumber plants two seasons in a year for ^{88}Sr approximately 14 times, which needed 7 years, followed by ^{133}Cs 26 times, which needed 13 years, and ^{88}Sr + ^{133}Cs 10 times, which needed 5 years, theoretically, to uptake all the ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs from the soil, respectively; when the ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs, concentration in the soil was 160 mg kg^{-1}, it needed to grow cucumber plants two seasons in a year for ^{88}Sr approximately 62 times, which needed 31 years, followed by ^{133}Cs 110 times, which needed 55 years, and ^{88}Sr + ^{133}Cs 48 times, which need 24 years, theoretically, to uptake all the ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs from the soil. Similar results were recorded in [45].

4.4. Physiological and Biochemical Response of Cucumber to ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs Stress

These results corroborate those reported by other studies [42,50]. Decreased chlorophyll contents with heavy metal stress may be due to the inhibition of enzymes responsible for chlorophyll biosynthesis and a strong production of reactive oxygen species [50]. Chlorophyll content was increased at 10 and 20 mg (Figure 2); however, it was reduced by increasing the concentrations of treatments. This phenomenon is due to excessive ^{88}Sr and ^{133}Cs, which significantly reduced the chlorophyll content compared to ^{88}Sr + ^{133}Cs. Similar results were obtained by other researchers who studied the impact of Sr on oilseed rape [46], *Amaranthus caudatus Linn* [51], maize [6], *Amaranthus mangostanus* L. [45], and *Raphanus sativus* L [42]; and the impact of Cs on *Nitella pseudoflabellata* [52], *Salix paraplesia* [53], *Spinacia oleracea* [54], *Amaranthus mangostanus* L. [45], and *Raphanus sativus* L. [42]. MDA is an important product of membrane lipid peroxidation, and it can indirectly reflect the degree of damage to the plant membrane system. In this experiment, MDA content slightly decreased at low ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs concentrations (10 and 20 mg kg^{-1}) and then increased at high concentrations (40, 80, and 160 mg kg^{-1}); this change trend indicated that low concentrations of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs had little destructive effects on cucumber membrane function, while the high ^{88}Sr concentration treatments led to impairment of cucumber membrane function. These MDA changes were similar to those found by [42,45]. The increased activity of antioxidant enzyme activities (CAT, POD, and SOD) indicated the activation of defense mechanisms against the ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs that induced oxidative stress in cucumber. In the present study, the activity of CAT, POD, and SOD increased gradually with increasing ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs concentrations from (10 to 160 mg) (Figure 3). Similarly, the Sr and Cs application resulted

in the increased activity of CAT, POD, and SOD in *Amaranthus mangostanus* L. [45,51], the activities of CAT and POD in *Amaranthus caudatus* L. [45], and CAT, POD, and SOD in *Raphanus sativus* L. [42].

5. Conclusions

The Sr and Cs contamination threats to the soil environment hence are liable to be removed. Phytoremediation is an effective tool to combat the hyper-accumulation of radionuclides and heavy metals in soil. The cucumber plant showed significantly lower growth under various concentrations of individual ^{88}Sr and ^{133}Cs treatments than did ^{88}Sr + ^{133}Cs combined. ^{133}Cs produced more loss in terms of growth and biomass production. The ^{88}Sr + ^{133}Cs when applied in combination showed significantly better results, indicating some mitigating effects. Shoot accumulation was high for ^{88}Sr + ^{133}Cs, while root accumulation was higher for ^{88}Sr than for ^{133}Cs. Similarly, the bioaccumulation and translocation of ^{88}Sr + ^{133}Cs was higher than of ^{88}Sr and ^{133}Cs treatments. This shows that ^{88}Sr + ^{133}Cs combined are absorbed and translocated at high levels and at the same concentrations and enhance growth and biomass production. It is well known that radionuclides and heavy metals will directly or indirectly cause molecular damage to plant cells through the outbreak of reactive oxygen species (ROS), and ROS will react with fatty acids, leading to lipid peroxidation and damage to biofilms [55,56]. One of the responses of plants to ROS is an increase in antioxidant enzyme activity, thereby protecting them from oxidative damage induced by various stresses [55]. Some plants' tolerance to heavy metal stress is related to the higher activity of antioxidant enzymes [57–59]. The ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs treatment concentrations sequentially induced some enzymes over 60 days of exposure, suggesting that this complex of antioxidant enzymes (CAT, POD, and SOD) work in combination to reduce the impact of toxicity of ^{88}Sr, ^{133}Cs, and ^{88}Sr + ^{133}Cs, especially in young leaves. It is concluded that cucumber reveals considerable phytoremediation capabilities due to the unique growth potential in contaminated substrate and is suitable for bioreclamation of degraded soils. The plant is especially applicable for efficient phytoextraction of ^{88}Sr + ^{133}Cs-contamination.

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