Biochemical Analysis and Toxicity Assessment of Utilization of Argon Oxygen Decarbonization Slag as a Mineral Fertilizer for Tall Fescue (Festuca arundinacea Schreb) Planting

Shuang Cai, Bao Liu *, Junguo Li *, Yuzhu Zhang, Yanan Zeng, Yajun Wang and Tianji Liu

Abstract: Argon oxygen decarbonization (AOD) slag refers to a byproduct of stainless steel (SS) production, which has caused considerable environmental stress. Finding an effective approach for recycling AOD slag is essential to environmental safety. In this work, batch leaching tests were carried out to explore the leaching behavior of AOD slag and soil. Pot experiments was conducted to analyze the fertilization effect of AOD slag for tall fescue (Festuca arundinacea Schreb) planting. The plant height, biomass, total root length (TRL), root surface area (RSA), root tips (RT), root hairs (RH)), chlorophyll content, malondialdehyde (MDA) content, and antioxidant enzyme activities of the tall fescue seedlings were measured. As indicated from the results, adding AOD slag into soil increased soil pH. The leaching concentration of Ca, Si, Al, Cr of the AOD slag was higher than the original soil, while that of Mg, Mn, and Fe was lower. Low addition rate (≤ 1%) of AOD slag fertilization was good for plant height, biomass, root growth, and chlorophyll synthesis, whereas high addition rate (≥ 2%) exerted an opposite effect. Elevating the rate of AOD slag fertilization increased the Cr accumulation in the tall fescue seedling that aggravated damage of reactive oxygen species (ROS). When the AOD slag fertilization was at a low rate (≤ 1%), ROS scavenging was attributed to the synergistic effects of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) defense systems, while at a high rate (≥ 2%) of AOD slag fertilization, scavenging of excessive ROS could be mainly due to the CAT defense system.

Keywords: AOD slag; mineral fertilizer; tall fescue; chromium accumulation

1. Introduction

The steel and iron industry produces slag such as blast furnace (BF) slag, ladle furnace (LF) slag, and stainless steel slag (SSS) during iron-making and steel-making processes. In 2020, the output of crude stainless steel (SS) in China was higher than 30 million tons, with a year-on-year increase of 2.51%. According to [1], 3 tons SS can produce 1 ton of SSS, the annual output of SSS exceeds 10 million tons in China. Currently, little SSS has been recycled to produce cement and concrete [2–5], and most are discharged and piled up without treatment, which causes waste of land and serious environmental pollution. Finding a good approach for SSS recycling is greatly conductive to the environmental safety.

The solid wastes have recently been reported to be utilized as a mineral fertilizer in agriculture [6–9]. Si-K fertilizer made from steel slag can effectively improve the agricultural soil fertility and promote the rape growth [10]. Preston et al. [9] showed that using steel mill slag as the Si source for melon (Cucumis melo L.) planting reduces the severity and incidence while improving fruit quality. Fonseca et al. [11] reported that adding metallurgical slag into soil increases the macronutrients in the shoot of grass, benefiting its growth. Based on smelting processes, SSS is composed of electric arc furnace (EAF) slag and argon oxygen decarbonization (AOD) slag. AOD slag contains CaO, SiO₂, MgO, FeO, and MnO, which
can provide micronutrients for plant growth. Therefore, the application of SSS as a mineral fertilizer in agriculture may be a feasible way for its utilization.

Cr content in AOD slag is commonly less than 1 wt.% [12]. When adding AOD slag into soil, Cr in AOD slag would be leached out as influenced by rainwater and absorbed by plant. Cr is a non-essential element for plant growth. Although the promotion of some plants’ growth at low Cr concentration was reported by Christou et al. [13], it is toxic at high concentration [14]. The high Cr concentration in plants can inhibit photosynthesis, trigger lipid peroxidation, and result in considerable damage to plant growth [15]. Like other heavy metals, Cr stimulates the generation of reactive oxygen species (ROS) such as H$_2$O$_2$, O$_3$, OH$^-$, etc. [16,17]. The overproduction of ROS would disrupt cell homoeostasis, damage photosynthetic pigments, and break DNA strands, inhibiting the growth of plants [18]. Plants would evolve complex antioxidant defense system to protect themselves against the ROS damage, generating enzymatic antioxidants including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), etc. To our knowledge, the effects of AOD fertilization on the growth of tall fescue (Festuca arundinacea Schreb) have not been understood. Moreover, the enzymatic antioxidants scavenge mechanism of ROS of the tall fescue is still unknown and need to be investigated.

Tall fescue, an important perennial cool-season forage and turfgrass, was selected as the test plant. As reported [19], the tall fescue is widely used in temperate regions throughout the world because of its good adaptability to drought, high temperature, and heavy metals. In this work, the feasibility of utilization of AOD slag as a mineral fertilizer for tall fescue planting was evaluated. The leaching behavior of AOD slag and soil was investigated by batch leaching tests. AOD slag and soil were fully mixed for pot experiments of tall fescue planting. The plant height, biomass, root properties, and chlorophyll content of the tall fescue seedling were measured. The effect of AOD slag fertilization on the Cr accumulation in the tall fescue seedling was explored for environmental safety. The enzymatic antioxidants scavenge mechanism of ROS of the tall fescue antioxidant was studied by measuring SOD, POD, and CAT activities.

2. Materials and Methods

2.1. AOD Slag

AOD slag was sourced from Qingshan Stainless Steel Plant in China. Before being used for pot experiments, the collected AOD slag was dried at 105 °C for 6 h and ground to particle sizes below 74 µm. The AOD slag was analyzed by X-ray fluorescence (XRF) to determine its chemical composition and shown in Table 1. The main chemical compositions of AOD slag were CaO, SiO$_2$, and MgO, accounting for 93.152 wt.% of the total compositions. Other oxides with low weight (wt.) contents of 1.722 wt.%, 0.358 wt.%, 0.212 wt.%, and 0.171 wt.% were Al$_2$O$_3$, Fe$_2$O$_3$, TiO$_2$, and MnO, which are the trace chemical compositions of the AOD slag. The weight content of Cr$_2$O$_3$ was 0.378 wt.%. Except for Cr$_2$O$_3$, there are almost no other heavy metal oxides in AOD slag.

| Oxide     | Content (wt.%) | Oxide     | Content (wt.%) |
|-----------|----------------|-----------|----------------|
| CaO       | 62.076         | MnO       | 0.171          |
| SiO$_2$   | 26.213         | SrO       | 0.024          |
| MgO       | 4.863          | ZrO$_2$   | 0.012          |
| Al$_2$O$_3$| 1.722         | Nb$_2$O$_5$| 0.007          |
| Fe$_2$O$_3$| 0.358         | SO$_3$    | 0.594          |
| Cr$_2$O$_3$| 0.378         | P$_2$O$_5$| 0.006          |
| TiO$_2$   | 0.212          | F         | 3.363          |

2.2. Batch Leaching Test

To investigate the leaching behavior of AOD slag and soil, batch leaching tests were performed. First, 90 g AOD slag or soil was added into 900 mL deionized water with a liquid
to solid ratio (L/S) of 10:1 in a conical flask. The flask was horizontally vibrated at 25 °C using the gas bath thermostatic vibrator. After leaching for 24 h, the leachate was sampled and filtered by a 0.45-µm membrane filter. The pH of the leachate was measured using the pH meter. The Ca, Si, Mg, Al, Fe, Mn, and Cr concentrations of the leachate were measured using Agilent ICPMS7800 inductively coupled plasma mass spectrometry (ICP-MS).

2.3. Pot Experiments

The tall fescue seeds were purchased from Linyi City, Shandong Province, China. The seed purity was ≥98.0%, the germination rate was ≥90.0%, and the water content was ≤8.0%. The soil used in this work was the peat matrix soil (Klasmann peat 876#, Germany). The detailed soil properties can be found in our previous study and shown in Table 2 [20]. The peat soil was dried by air and screened by using a 2 mm sieve. Undersized peat soil was fully mixed with AOD slag. The addition rates of AOD slag were 0%, 0.25%, 0.5%, 1%, 2%, 4%, 8%, and 16% (w/w), respectively. A total of 30 tall fescue seeds were selected and sowed in the plastic pot filled with soil–slag mixture (0.15 kg per pot). It should be repeated three times in each AOD slag addition rate to ensure the reliability. During the growth of tall fescue, the light and dark hours were set as 16 h and 8 h, respectively, and the corresponding temperatures were 25 °C and 20 °C, respectively. Each pot was watered every other day using deionized water to 70% of field capacity. The tall fescue seedlings were thinned to 10 per pot 7 days after emergence and were harvested after 30 days. The plant height, biomass, root properties, malondialdehyde (MDA) content, superoxide dismutase (SOD) activity, peroxidase (POD) activity, catalase (CAT) activity, and chlorophyll content were determined.

Table 2. Physicochemical properties of the original soil.

| Type               | Value      | Element | Content (mg kg⁻¹) |
|--------------------|------------|---------|-------------------|
| pH                 | 6.13 ± 0.06| Ca      | 11,843.19 ± 493.69|
| EC (mS cm⁻¹)       | 0.50 ± 0.04| Si      | 1335.44 ± 39.38   |
| OM (g kg⁻¹)        | 601.17 ± 14.51| Mg | 1236.86 ± 156.10  |
| Total N (g kg⁻¹)   | 7.06 ± 0.73 | Al      | 376.05 ± 9.36     |
| Total P (g kg⁻¹)   | 0.72 ± 0.09 | Fe      | 695.05 ± 19.69    |
| Total K (g kg⁻¹)   | 1.01 ± 0.12 | Mn      | 56.28 ± 10.09     |
| Available N (mg kg⁻¹)| 337.54 ± 6.22| Ti | 10.29 ± 0.77      |
| Available P (mg kg⁻¹)| 80.44 ± 2.09| Cr      | 3.52 ± 0.26       |
| Available K (mg kg⁻¹)| 589.00 ± 9.35| Cu | 0.57 ± 0.08       |
|                    |            | Zn      | 18.02 ± 1.94      |
|                    |            | Ni      | 5.21 ± 0.70       |
|                    |            | Cd      | 0.08 ± 0.01       |
|                    |            | Hg      | 0.07 ± 0.02       |
|                    |            | Pb      | 3.86 ± 0.48       |

2.4. Measurements

X-ray diffraction (XRD) analysis was performed to detect the mineralogical composition of the AOD slag. XRD measurements were conducted by a Brucker D8 Advance X-ray diffractometer. The scanning rate was 10° 20 min⁻¹, the 2θ range was 10–90°. The morphology of the AOD slag was determined by a SU8020 field emission-scanning electron microscopy (FESEM).

After harvesting, the above-ground parts and the root of the tall fescue seedling were cleaned with deionized water and dried. The total root length (TRL), root surface area (RSA), root tips (RT), and root hairs (RH) were scanned by an EPSON-10000XL root scanner, and the obtained images were analyzed by winPHIZO. The chlorophyll content, MDA content, SOD, CAT, and POD activities were measured using the above-ground parts of the fresh sample. Chlorophyll content in the tall fescue seedling was determined by acetone extraction spectrophotometry method [21]. MDA content was detected by referring to the method introduced by Chen et al. [22]. SOD activity was determined by monitoring the
photochemical reduction inhibition of p-nitroblue tetrazole with the method described by Gianopoli and Ries [23]. The CAT activity and the POD activity were identified by decomposition of H$_2$O$_2$ and the guaiacol oxidation method, respectively [24]. The chlorophyll content, MDA content, and antioxidant enzyme activities were all expressed in fresh weight (FW).

The accumulation of Cr in the tall fescue seedling was determined by undertaking the dried above-ground parts as the testing samples. The obtained tall fescue seedlings were washed with deionized water first, then dried at 60 °C so that the weight was constant weight. Using the Master-18 microwave digestion system, the seedlings were digested with nitric acid solution. After that, the obtained solution was diluted, and the Cr concentration of the tall fescue seedling was measured by ICP-MS. The calculated Cr accumulation content of the tall fescue seedling was expressed in dry weight (DW).

2.5. Statistical Analysis

The obtained physiological and biochemical parameters were statistically analyzed by SPSS 25.0. All data were expressed in the form of mean ± standard deviation (SD) of three replicates. For multiple comparisons, the significant difference between the addition rates were identified using one-way ANOVA followed by Duncan’s test at a level of $p < 0.05$.

3. Results and Discussion

3.1. Minerals and Morphology of AOD Slag

Figure 1 shows the XRD patterns of the AOD slag. XRD analysis shows that the AOD slag had a large amount of silicates (e.g., dicalcium silicate (Ca$_2$SiO$_4$), augite (Ca(Mg$_{0.85}$Al$_{0.15}$)(SiO$_4$)$_2$), and merwinite (Ca$_3$Mg(SiO$_4$)$_2$). In addition, there were also some other minerals in the AOD slag such as fluorite (CaF$_2$) and periclase (MgO). Since CaO and SiO$_2$ were the main chemical composition, the dicalcium silicate was identified as the major mineral in AOD slag. Similar results were reported by Moon et al. [25]. They indicated that the dicalcium silicate with the weight content of 38.1 wt.% was the major mineral of AOD slag. Zuo et al. [26] also suggested that the dominant minerals in AOD slag were gamma dicalcium silicate, beta dicalcium silicate, and periclase. Due to the low content of Cr$_2$O$_3$, the Cr-bearing phase was not detected by XRD analysis. According to the literature [27–29], Cr is commonly found in spinel (e.g., FeCr$_2$O$_4$ and MgCr$_2$O$_4$) or solid dissolved in silicates and glass phase. As reported by Gu et al. [30], 70% Cr in SSS existed in the form of oxide. Ji et al. [31] indicated that Cr was mainly contained in the spinel phase, and a portion of Cr was found in the metallic phase and periclase phase of the SSS with 3.93 wt.% Cr$_2$O$_3$. Figure 2 depicts the morphology of the AOD slag powder. As observed, the AOD slag contained the particles with a noticeable angled edge that is similar to the ordinary steel slag. Most of the AOD slag particle sizes were lower than 50 μm.

![Image](image_url)
3.2. Leaching Behavior of AOD Slag and Soil

Figure 3 depicts the leaching behavior of the AOD slag and soil. As exhibited in Figure 3a, pH levels of the AOD slag and soil leachates were 12.15 and 5.81, respectively. The pH of the AOD slag was much higher than the soil. This indicates that adding AOD slag into soil would significantly enhance the soil pH. Figure 3b depicts the leaching concentrations of the elements in AOD slag and soil. As observed, the leaching concentrations of Ca, Si and Al of the AOD slag were higher than that of the soil, while the leaching concentrations of Mg, Mn, and Fe of the AOD slag were lower. For the heavy metal Cr, the Cr leaching concentration of the AOD slag was 0.077 mg L\(^{-1}\) which is 7 times higher than the soil. Based on the results of batch leaching tests, the utilization of AOD slag as a mineral fertilizer may be unjustified. Although adding AOD slag into soil can improve Si content, the low Mg, Fe, and Mn concentration, the high pH, and the high Cr concentration is likely to be harmful to the growth of tall fescue seedling [32–34].

Figure 3. Leaching behavior of the AOD slag and soil, (a) pH, (b) elemental leaching concentrations. The error bars indicated SD.

3.3. Effects of AOD Slag Fertilization on Growth of Tall Fescue Seedling

The effects of AOD slag fertilization on the plant height and biomass of tall fescue were shown in Figure 4. With increased rate of AOD slag, both plant height and biomass increased and then decreased. The maximum values of plant height and biomass could be obtained at 0.5% of the addition rate. Compared with the sample grown in the original soil, the plant height increased by 17.8%, while the biomass increased by 120.7%. Low rates (≤1%) of AOD slag were beneficial to the growth of tall fescue seedling. The reductions in plant height and biomass were observed at ≥2% of addition rate. The tall fescue could not grow well in the soil–slag mixture when it was ≥4%. After the addition rate reached 16%, the plant height and biomass declined by 52.2% and 93.8%, respectively, compared with the original soil.
The morphology of the root was analyzed, and the resulting images are shown in Figure 5. In addition, the properties of the root were also analyzed, and the specific analysis data are given in Table 3. The variation of the TRL, RSA, RT, and RH exhibited a similar trend to the plant height and biomass. At the best AOD slag addition rate (0.5%), the TRL, RSA, RT, and RH increased by 40.5%, 26.4%, 62.1%, and 89.2%, respectively, compared with the sample grown in the original soil. The adverse effects of AOD slag fertilization on these root properties were clear when the addition rate was $\geq 2\%$. With the addition rate increased to 16\%, the TRL, RSA, RT, and RH declined by 65.4\%, 59.2\%, 54.0\%, and 66.7\%, respectively.

Figure 4. Effects of different rates of AOD slag on plant height and biomass 30 days after germination, (a) plant height, (b) biomass. The error bars indicate SD, and the lowercase letters mean statistical significance with $p < 0.05$.

Figure 5. Variation of root morphology of the tall fescue seedlings 30 days after germination, (a) 0\%, (b) 0.25\%, (c) 0.5\%, (d) 1\%, (e) 2\%, (f) 4\%, (g) 8\%, (h) 16\%.
As mentioned above, the pH of AOD slag was much higher than the soil. The addition of AOD slag into soil would improve soil pH. Our previous study [20] showed that the soil pH increased from 5.81 to 9.73 with increasing AOD slag addition rate from 0% to 32%. According to the research by Li et al. [35], the tall fescue presents the best growth status when the pH increased from 5.81 to 9.73 with increasing AOD slag addition rate from 0% to 32%. The release of Ca and Si from the AOD slag could increase the micronutrients for the growth of the tall fescue seedling. Therefore, low addition rates (≤1%) contributed to plant height, biomass, and root growth. The further increase of addition rate would result in high soil pH levels and low Mg, Fe, and Mn concentrations that were harmful to the growth of tall fescue seedlings. The absorption and translocation of the mineralogical ions from soil to plant are mainly influenced by the root system [36]. The reductions in TRL, RSA, RT, and RH inhibited the absorption of mineralogical ions, hindering the growth of the tall fescue seedling. In addition, higher rate of AOD slag would also elevate Cr content which is also harmful to the growth of the tall fescue seedling.

### 3.4. Effects of AOD Slag on Chlorophyll Content

Chlorophyll is a photosynthetic agent which can help plants to absorb light energy and convert it into chemical energy. Figure 6 shows the effects of AOD slag fertilization on chlorophyll content in tall fescue seedlings. When the AOD slag addition rate increased from 0% to 1%, the chlorophyll level was continuously elevated. The chlorophyll content started to reduce at the rate >1%. Compared with the condition in original soil, the chlorophyll level increased by 17.6% at 1% while it decreased by 20.9% at 16%.

![Figure 6](image_url)

**Figure 6.** Effects of different rates of AOD slag on chlorophyll content 30 days after germination. The error bars indicate SD and the lowercase letters mean statistical significance with *p* < 0.05.
The synthesis of chlorophyll is mainly influenced by Mg, Fe, and Mn contents of the tall fescue [37–39]. As mentioned above, the growth of the root was promoted with the AOD slag addition rate ≤1%, while it was inhibited with the addition rate >1%. The Fe, Mg, and Mn ions were mainly absorbed by the root system of the tall fescue. The increase of TRL, RSA, RT, and RH benefited the synthesis of chlorophyll through the strong absorption of Fe, Mg, and Mn ions. The inhibition of root growth at high rate of AOD slag reduced the chlorophyll level of the tall fescue seedling. In addition, higher AOD slag addition rates caused an elevated pH level, at which the leached Fe, Mg, and Mn ions would be precipitated as FeOOH, Mg(OH)₂, and MnOOH, respectively, inhibiting absorption of these ions by the tall fescue [40].

3.5. Cr Accumulation in Tall Fescue Seedling

Figure 7 illustrates the changes of Cr accumulation content of the tall fescue seedlings caused by adding different contents of AOD slag. As shown in Figure 7, the Cr accumulation content of the tall fescue seedling was elevated with continual increase of rate of AOD slag. Increased Cr accumulation was inapparent with the rate range of 0.25–2%, while the Cr accumulation content was elevated obviously when the addition rate was >2%. Compared with the original soil, Cr accumulation increased by 34.8% and 126.1% with the AOD slag addition rates of 2% and 16%, respectively. Cr is toxic for plants. Numerous studies have indicated that quantity of Cr accumulation in plants may delay the germination, decrease seed germination rate, decrease TRL, damage RH formation, reduce chlorophyll content, inhibit plant photosynthesis, etc. [41–44]. In turn, the delay and decrease of plant growth can be mainly attributed to the overproduction of reactive oxygen species (ROS) and the inhibition of the enzyme activity [44].

![Figure 7. Effects of different rates of AOD slag on Cr accumulation in the tall fescue seedlings 30 days after germination. The error bars indicate SD and the lowercase letters mean statistical significance with \( p < 0.05 \).](image-url)

Figure 8 shows the variations of MDA content, SOD, POD, and CAT activities under the effects of AOD slag fertilization. MDA is decomposed from polyunsaturated fatty acid hydroperoxides, which can be used to estimate the damage extent of ROS to membrane lipids [45]. As shown in Figure 8a, the MDA contents were a little lower at the rates of 0.25–2% than the original soil. This indicates that low addition rates of AOD slag fertilization are not harmful to the tall fescue seedling. MDA content increased obviously when it was >2%. Compared with the status in the original soil, the MDA content increased by 79.2% at 16% of AOD slag addition rate. This suggests that serious ROS damage to the tall fescue seedling occurred at high AOD slag addition rates. The variation trend of MDA content was similar to that of the Cr accumulation content of the tall fescue seedling, indicating that the ROS damage was mainly induced by Cr.
AOD slag fertilization could accelerate plant height, biomass, and root growth, whereas the synergistic effects of SOD, POD, and CAT defense systems. High addition rates (>1%) of AOD slag fertilization could accelerate plant height, biomass, and root growth, whereas high addition rates (≥2%) showed an opposed effect. Increasing the addition rate of AOD slag fertilization could elevate the Cr accumulation content of the tall fescue seedlings, which was harmful to the seed germination, root growth, and chlorophyll synthesis. The extent of ROS damage was significantly enhanced when the tall fescue seedling, which was harmful to the seed germination, root growth, and chlorophyll synthesis. The extent of ROS damage was significantly enhanced when the planted AOD slag was greater than 1%. The scavenging of the excessive ROS could be attributed to the synergistic effects of SOD, POD, and CAT defense systems. Remarkably, high addition rates (≥2%) inhibited the SOD and the POD defense systems. Therefore, when the added AOD slag was ≤1%, the scavenging of the excessive ROS could be attributed to the synergistic effects of SOD, POD, and CAT defense systems of the tall fescue seedling. Remarkably, high addition rates (≥2%) inhibited the SOD and the POD defense systems. The CAT defense system played a crucial role in eliminating ROS damage at high AOD slag addition rates.

4. Conclusions

In this work, the fertilization effects of AOD slag on tall fescue seedling were investigated. Adding AOD slag into soil would increase soil pH, Ca, Si, Al, and Cr leaching concentrations, and decrease Mg, Mn, and Fe leaching concentrations. Low rates (≤1%) of AOD slag fertilization could accelerate plant height, biomass, and root growth, whereas high addition rates (≥2%) showed an opposed effect. Increasing the addition rate of AOD slag fertilization could elevate the Cr accumulation content, resulting in ROS damage to the tall fescue seedling, which was harmful to the seed germination, root growth, and chlorophyll synthesis. The extent of ROS damage was significantly enhanced when the added AOD slag was >2%. At low rates (≤1%), the elimination of ROS can be attributed to the synergistic effects of SOD, POD, and CAT defense systems. High addition rates (≥2%) of AOD slag inhibited SOD and POD defense systems. Scavenging of the excessive ROS could be mainly due to the CAT defense system. We concluded that it is possible to use AOD slag as a mineral fertilizer for tall fescue planting. For the plant growth perspective,
the addition rate of AOD slag as a mineral fertilizer for tall fescue planting should not ≥1%, and the best addition rate was 0.5% (≈450 kg ha⁻¹). However, adding AOD slag into soil would increase Cr content of the original soil, resulting in soil contamination. The risks of AOD slag fertilization on soil safety should be further evaluated.

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**Data Availability Statement:** The data presented in this study are available on request from the corresponding author. The data are not publicly available due to the data also forms part of an ongoing study.

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