

Enhanced Cd Phytoextraction by *Solanum Nigrum* L From Contaminated Soils Combined With the Application of N Fertilizers and Double Harvests

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Abstract

It is very important to increase phytoremediation efficiency in practice in suitable climatic conditions for plant growth by multiple harvests. *Solanum nigrum* L. is a Cd hyperaccumulator. In present experiment, after applying different types of N fertilizers (NH_4HCO_3 , NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$, $\text{CH}_4\text{N}_2\text{O}$), root and shoot biomasses and Cd phytoextraction efficiency of *S. nigrum* effectively improved ($P < 0.05$), whereas shoot biomasses of *S. nigrum* harvested at the first florescence stage plus the amounts at the second florescence stage were higher than those at the maturation stage, which indicated that *S. nigrum* Cd phyto-accumulation efficiency was higher in double harvests at florescence stages compared to a single harvest due to the lack of a clear change in Cd concentration ($P < 0.05$). The pH value and extractable Cd contents showed no changes, regardless whether N fertilizer was added or not at different growth stages. In addition, after N fertilizer supply, H_2O_2 and MDA contents in *S. nigrum in vivo* were lower compared to CK; Similarly, the concentration of proline was decreased as well ($P < 0.05$). As one of the antioxidant enzymes, CAT activity in *S. nigrum* shoots, harvested at different growth periods after 4 types of N fertilizer application, obviously decreased, while POD and SOD activities increased ($P < 0.05$). Our study demonstrated that $(\text{NH}_4)_2\text{SO}_4$ treatment exerted the most positive effect and the $\text{CH}_4\text{N}_2\text{O}$ the second-most positive effect effect on *S. nigrum* Cd phytoremediation efficiency in double harvests at florescence stages and the growth conditions were better than others.

1. Introduction

Cadmium (Cd), as one of the most common heavy metals in the environment, has extremely toxic biochemical characteristics, even at low levels (Yuan et al., 2021), and the growth, development and yields of crops will be seriously threatened and would occur a series of damages at morphological, physiological and biochemical levels in plant cells and tissues, even harm human health in the food chain (Zhou and Song, 2004). Phytoremediation, a novel biological technique that utilizes plant materials – hyperaccumulators to remediate soil contaminated by heavy metals – is superior to many other physical and chemical measures due to its high efficiency, environmental friendliness, long term availability and low price (Feng et al., 2019; Zhou et al., 2020). Usually, hyperaccumulators showed 4 basic characteristics. Firstly, concentrate heavy metals in plants should be than that in soils; secondly, the shoot concentration standard of Zn is $10,000 \text{ DW mg kg}^{-1}$, the Cu, Pb, Ni and Co is $1000 \text{ DW mg kg}^{-1}$ and the Cd is $100 \text{ DW mg kg}^{-1}$; the third, the accumulation concentration of heavy metals in aboveground part is higher than in the root, while the plants grow well and no obviously toxic symptoms appear (Zhou and Song, 2004). Of which, some hyperaccumulators includes Cd hyperaccumulators *Noccaea caerulea* (Rees et al., 2015), *Solanum nigrum* L. (Dou et al., 2020) and *Viola baoshanensis* (Wu et al., 2010), Cr hyperaccumulator *Leersia hexandra* Swartz (Liu et al., 2011), Zn hyperaccumulator *Sedum alfredii* Hance (Guo et al., 2020; Wu et al., 2020), Ni hyperaccumulator *Alyssum murale* (Bani et al., 2018) and As hyperaccumulator *Pteris vittata* (Singh et al., 2016; Xiang et al., 2020), etc. However, for most hyperaccumulators, the disadvantages of slow-growth property, low-weight yield and geographical constraints will limit their potential for amending a large scale of heavy metals-polluted arable land (Nie

et al., 2016), and thus they cannot achieve better remediation efficiency in practice. The addition of environmentally friendly additives, such as inorganic, organic fertilizers and bio-fertilizers (Wang et al., 2019), CO₂ gas supply (Li et al., 2014) and intercropping (Wang et al., 2015) could improve phytoextraction efficiency by hyper- and intermediate accumulators owing to the increased biomasses and/or the improved Cd shoot concentration.

Nitrogen (N) is an essential major element, which improves soil fertility and biochemical properties and increases crop biomass yields (it is involved in many metabolic processes, including the biosynthesis of amino acids, nucleic acids and proteins in plants and improves the resistance to abiotic and biotic stresses. For non-hyperaccumulators, Most research indicated that N application reduce the production of large amounts of reactive oxygen species (ROS) *in vivo* induced by heavy metal Cd stress, such as superoxide radicals (O₂^{•-}), hydrogen peroxide (H₂O₂) and hydroxyl radicals (•OH) and maintain the balance of the generation and elimination of ROS so as to withstand the secondary oxidative stress (Abid et al., 2019). Simultaneously, it generates a synergistic effect on sophisticated antioxidant defense system to increase the contents of low molecular weight non-enzymatic antioxidants such as proline, GSH and AsA, and improve the activities of antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and another four enzymes in AsA-GSH recycle system composed of Ascorbic acid peroxidase (APX), glutathione reductase (GR), dehydroascorbic acid reductase (DHAR) and monodehydroascorbic acid reductase (MDAR) to alleviate Cd toxicity in plants, and thereby protecting the plant itself (Yu et al., 2019). These biochemical indexes are generally recognized as the most sensitive and typical parameters to evaluate plant tolerance to heavy metals (Zhang et al., 2014; Hui et al., 2015) .

According to the above conclusions, we realized that N fertilizer could mitigate the adverse effect of Cd and decrease Cd uptake by non-hyperaccumulators. Conversely, some research studying N application showed that N fertilizer supply could enhance phytoremediation of Cd-contaminated soils by increasing the shoot biomass yields and no decreasing Cd uptake by hyperaccumulators (Wei et al., 2015; Yang et al., 2019). That is to say, N application had different effects on Cd bioaccumulation and antioxidant defense mechanism between hyperaccumulator and non-hyperaccumulator. Additionally, many articles have reported that hyperaccumulators could exert themselves unique detoxification mechanisms, such as Cd-PCs chelate synthesis, vacuolar compartmentalization and powerful GSH pool to scavenge the ROS induced by heavy metal (HMs) stresses. This is in agreement with the consequence described by Sun et al., who in-depth elucidated that the antioxidative defense mechanism rather than PCs enhancement in hyperaccumulator *in vivo* could effectively play a crucial role in resisting against ROS induced by Cd stress (Sun et al., 2007). Obviously, the previously published studies are incompatible, and thus it is a necessity to specifically explore the Cd phytoremediation efficiency and a relationship between N application and antioxidant defense mechanism including non- and enzyme antioxidants in hyperaccumulators subjected to the Cd stress.

Taking into consideration the preceding results, we selected the newly found Cd hyperaccumulator *S. nigrum* as our present experiment materials, which has attracted widespread attention due to its

distinctive advantages of rapid growth rate, viability and environmental suitability (Wei et al., 2015), and the objective of our study is to investigate that which one or two fertilizers could obtain maximum Cd phytoaccumulation in different growth stages and induce the strongest antioxidant enzyme activities so as to maintain the available growth stage under different types of N fertilizers. Meanwhile, we compared single and double crop harvests, thereby achieving optimization of Cd removal from contaminated soil.

2. Materials And Methods

2.1 Basic physicochemical properties of soil and the pot experiment

The used soil sample was collected from local agricultural field, which is meadow brown soil and the type of soil sample belonged to neutral soils. Seeds of *S. nigrum* were also collected from the local field. The soil properties, local climate conditions are all consistent with the previous article (detail data was omitted due to Crossref Similarity Check) (Yang et al, 2019).

Cd was spiked in distilled water with no Cd detected in the form of $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ and the Cd concentration in all treatment groups was 2 mg kg^{-1} . Compared with the Soil Environmental Quality standard in China (GB-15618-2018), the pollution level was moderate (SEQ, 2018). The pure nitrogen fertilizers used (NH_4HCO_3 , NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$ and $\text{CH}_4\text{N}_2\text{O}$) were purchased on the local market and mixed with added Cd together. The detail designed was shown in Table 1. Soil samples (2.5 kg of dry mass) were put in plastic pots and equilibrated for 2 months.

Six *S. nigrum* seedlings with four leaves and uniform height were transplanted from the seedling tray into each pot and each treatment was repeated 3 times. Plants were irrigated with tap water twice per day to maintain approximately 80% soil moisture content. Single cropping plants were harvested at maturity, while double cropping plants were harvested at the first and second florescence stage (Table 1), i.e. the first round harvest at florescence stage (the first florescence stage), and then transplanted *S. nigrum* seedling until florescence stage to be harvested (the second florescence stage). The maturation stage (118 days) was roughly equal to from seedling to the first florescence stage (59 days) plus the seedling to the second florescence stage (59 days) in one year.

2.2 Sample determination

Plant biomass and Cd concentration determination were same as the previous article and all detail information were omitted due to Crossref Similarity Check (Yang et al, 2019). Simply, the plant samples were divided into roots and shoots, and dried in the oven. The available Cd in the soil was extracted with 1 mM MgCl_2 . Cd concentrations in plant and soil samples were digested using concentrated HNO_3 and HClO_4 , and determined by AAS (Hitachi 180). The certification standard reference material (NIST SRM 1547, peach leaves) was used for quality control. Likewise, organic matter, nitrogen and extractable P contents were measured using a method described by Lu (Lu, 2000). The pH value was determined by a

pH meter (PHS-3B) and the soil to water ratio was 1:2.5 (v/w) (Wei et al., 2015). MDA (malondialdehyde) concentration was determined as described by Zhu et al. (Zhu et al., 2019). H_2O_2 content was measured using the previous method (Jiang and Zhang, 2001), and proline content was determined according to Bates et al. (Bates et al., 1973). CAT activity was measured by a modified method (Hasanuzzaman et al., 2011). SOD (Superoxide dismutase) and POD (peroxidase) activities were determined according to the provided references (Han et al., 2008). All treatments were performed in three replicates.

2.3 Data processing and Statistical analysis

Data processing and standard deviation (SD) calculations were performed using Excel. Means of different treatments were compared using one-way ANOVA with DPS software. For post hoc test, LSD multiple comparison was performed and the significance level was $p < 0.05$ based on the assumption of normal distribution and homogeneity of variance (Ma, 1990; Yang et al., 2019).

3. Results

3.1 Effects of different types of N fertilizers on shoot phytoextraction of Cd in *S. nigrum*

As seen in Table 2, under single and double harvests of *S. nigrum* demonstrated that there was no significant change in Cd concentration in roots and above-ground parts (shoots) of *S. nigrum* after applying different fertilizers (NH_4HCO_3 , NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$ and $\text{CH}_4\text{N}_2\text{O}$) compared to CK with no fertilizer addition ($p < 0.05$).

Nevertheless, the analysis of the single and double harvests experiments showed that Cd phytoremediation efficiency ($\mu\text{g plant}^{-1}$) in the shoots after application of 4 types of nitrogen fertilizers (NH_4HCO_3 , NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$ and $\text{CH}_4\text{N}_2\text{O}$) significantly increased compared to CK ($p < 0.05$). When *S. nigrum* were harvested at maturation stage, N additions significantly increased Cd phytoremediation efficiency compared to the CK ($17.66 \mu\text{g plant}^{-1}$), for NH_4HCO_3 increased by 1.20-fold (F3), NH_4Cl increased by 1.14-fold (F6), $(\text{NH}_4)_2\text{SO}_4$ increased by 1.88-fold (F9) and $\text{CH}_4\text{N}_2\text{O}$ increased by 1.84-fold (F12), respectively.

When *S. nigrum* were harvested at the first and the second florescence stages, the Cd phytoremediation efficiency ($\mu\text{g plant}^{-1}$) in the shoots were basically same (F2 and F3, F5 and F6, F8 and F9, F11 and F12), and their averages were $24.16 \mu\text{g plant}^{-1}$, $25.61 \mu\text{g plant}^{-1}$, $34.01 \mu\text{g plant}^{-1}$ and $33.03 \mu\text{g plant}^{-1}$, i.e. significantly increased by 36.8 %, 45.0 %, 92.6 % and 87.0 %, respectively compared to the CK.

In one year, the Cd phytoremediation efficiency ($\mu\text{g plant}^{-1}$) in the shoots of the first florescence stages plus the second florescence stages (double harvest) were $48.32 \mu\text{g plant}^{-1}$ (F1 + F2), $51.31 \mu\text{g plant}^{-1}$ (F4 + F5), $68.02 \mu\text{g plant}^{-1}$ (F7 + F8) and $66.60 \mu\text{g plant}^{-1}$ (F10 + F11), i.e. significantly increased by 1.74-fold (NH_4HCO_3), 1.90-fold (NH_4Cl), 2.85-fold ($(\text{NH}_4)_2\text{SO}_4$) and 2.77-fold ($\text{CH}_4\text{N}_2\text{O}$) compared to the CK,

respectively. Obviously, the Cd phytoremediation efficiency ($\mu\text{g plant}^{-1}$) in the shoots of double harvest increased by 24.3 % (F1 + F2 vs F3), 35.7 % (F4 + F5 vs F6), 33.8 % (F7 + F8 vs F9), 32.3 % (F10 + F11 vs F12), respectively.

3.2 Effects of different types of N fertilizers on root and shoot biomasses in *S. nigrum*

As shown in Table 3, dry weights of roots and above-ground plant parts of *S. nigrum* markedly increased ($p < 0.05$) in all treatment groups after applying the same amount of four types of N fertilizers (NH_4HCO_3 , NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$ and urea ($\text{CH}_4\text{N}_2\text{O}$)); dry weights of roots and above-ground parts were: $0.41 \text{ g plant}^{-1}$, $1.84 \text{ g plant}^{-1}$, $0.42 \text{ g plant}^{-1}$, $1.86 \text{ g plant}^{-1}$, $0.69 \text{ g plant}^{-1}$, $2.41 \text{ g plant}^{-1}$ and $0.70 \text{ g plant}^{-1}$, $2.41 \text{ g plant}^{-1}$, respectively, and in comparison to CK, they were increased by 1.64-, 1.15-, 1.66-, 1.18-, 3.43-, 1.81-, and 3.47-, 1.81-fold, respectively ($p < 0.05$). Under four types of N fertilizer (NH_4HCO_3 , NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$ and urea ($\text{CH}_4\text{N}_2\text{O}$)) treatments, our study confirmed that root and shoot biomasses harvested at the mature stage were higher than those at the first and second florescence stages. In addition, we calculated that total dry biomasses (g plant^{-1}) of the above-ground *S. nigrum* parts harvested at the first and second florescence stages using double cropping in one year were much larger than those harvested at the maturation stage using a single cropping per year; of all treatments, $(\text{NH}_4)_2\text{SO}_4$ and urea ($\text{CH}_4\text{N}_2\text{O}$) treatment groups obtained the highest shoot biomasses, and urea ($\text{CH}_4\text{N}_2\text{O}$) treatment showed the second (Table 3), i.e. the change trends of shoot biomasses were same as that of the Cd phytoremediation efficiency ($\mu\text{g plant}^{-1}$) (Table 2).

3.3 Effects of different types of N fertilizers on H_2O_2 and MDA contents in *S. nigrum* shoots

Table 4 shows that compared to CK without the addition of fertilizers, H_2O_2 (mg g^{-1}) and MDA ($\mu\text{mol g}^{-1}$) contents in *S. nigrum* shoots with four types of N fertilizer (NH_4HCO_3 , NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$ and urea ($\text{CH}_4\text{N}_2\text{O}$)) supply markedly decreased ($p < 0.05$), of which at the maturation stage, H_2O_2 contents (mg g^{-1}) decreased by 16.55%, 17.27%, 20.14% and 17.99%, respectively ($p < 0.05$). Similarly, MDA content ($\mu\text{mol g}^{-1}$) decreased by 4.20%, 3.94%, 3.67% and 4.20%, respectively, compared to CK ($p < 0.05$).

In addition, H_2O_2 and MDA contents in *S. nigrum* shoots harvested at the first and second florescence stages were the lowest.

3.4 Effects of different types of N fertilizers on proline concentration and the activity of CAT, POD and SOD in *S. nigrum* shoots

Figure 1(a) demonstrates that the addition of different N fertilizers ($(\text{NH}_4\text{HCO}_3$, NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$ and urea ($\text{CH}_4\text{N}_2\text{O}$)) at different growth stages resulted in a significant decrease in antioxidant proline contents (mg g^{-1}) and catalase (CAT) activities ($\text{U g}^{-1} \text{min}^{-1}$) in *S. nigrum* shoots in comparison to CK with no fertilizer addition ($p < 0.05$); at the maturation stage, the proline contents (mg g^{-1}) decreased by 13.63 %, 14.56 %, 15.10 % and 15.34 %, respectively; CAT activities ($\text{U g}^{-1} \text{min}^{-1}$) decreased by 6.64%, 6.17%, 5.38% and 7.54%, respectively, compared to CK ($p < 0.05$). Additionally, proline contents and CAT activities were the lowest at the first and second florescence stages (Fig. 1 (a,b)).

At the same time, we analyzed another two types of enzymes, i.e. peroxidase (POD) and superoxide dismutase (SOD). The results showed that after the application of different fertilizers, POD ($\text{U g}^{-1} \text{min}^{-1}$) and SOD activities (U g^{-1}) were markedly increased in comparison to CK with no fertilizer addition ($p < 0.05$); at the maturation stage, POD activities ($\text{U g}^{-1} \text{min}^{-1}$) increased by 12.20%, 11.35%, 11.80% and 11.39%, respectively; SOD activities (U g^{-1}) increased by 29.55%, 27.95%, 28.62% and 29.95%, respectively (Fig. 1(c,d)).

In addition, we analyzed the antioxidant system of *S. nigrum in vivo* at the first and second florescence stages and found POD and SOD activities in *S. nigrum* shoots were the highest (Fig. 1(c,d)).

3.5 Effects of different types of N fertilizers on extractable Cd concentration in *S. nigrum*

As shown in Fig. 2, the addition of four types of N fertilizers (NH_4HCO_3 , NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$ and urea ($\text{CH}_4\text{N}_2\text{O}$)) did not cause any significant difference in extractable Cd concentration in soil planted with *S. nigrum* harvested at different growing periods when compared to CK and a treatments were in the range of 1.11–1.15 mg kg^{-1} ($p < 0.05$).

3.6 Effects of different types of N fertilizers on soil pH in *S. nigrum* cultivation

Comparing the one-stage and two-stage phytoremediation Cd experiment involving *S. nigrum*, we found that the pH value in the rhizosphere soil in *S. nigrum* cultivation under all N fertilizer treatment groups (NH_4HCO_3 , NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$ and urea ($\text{CH}_4\text{N}_2\text{O}$)) did not differ significantly compared to control with no fertilizer supply and ranged from 6.60 to 6.70 ($p < 0.05$).

4. Discussion

4.1 Effects of different fertilizers on *S. nigrum* Cd phytoextraction in relation to single and double harvests

In a previous experiment, Wei et al (2006) showed that the Cd hyperaccumulator *S. nigrum* could accumulate Cd from slightly to moderately Cd-contaminated soil by double harvesting during the growing season, thereby showing an enormous potential for improving Cd accumulation efficiency in

practice (Wei et al., 2006), which was consistent with our finding. We demonstrated that the addition of different N fertilizers significantly enhanced total Cd phytoremediation efficiency ($\mu\text{g plant}^{-1}$) of *S. nigrum* under all treatments due to the significantly increased root and shoot biomasses (g plant^{-1}) and maintained Cd concentration in *S. nigrum* (Table 2, 3). We obtained higher shoot biomasses in double harvests compared to single harvests (Table 2). In contrast, other researchers conducted an experiment, which demonstrated that NH_4NO_3 and $\text{Ca}(\text{H}_2\text{PO}_4)_2$ application had no effect on biomass increase, while Cd concentrations significantly decreased compared to unfertilized plants (Ji et al., 2011). These authors concluded that the reason was the formation of metal-phosphate chelate, which reduced the solubility and mobility of heavy metal in soil. Similar to the above results, Fässler et al. (2010) also reported that $(\text{NH}_4)_2\text{SO}_4$ had no obvious effect on Cd accumulation by maize (Fässler et al., 2010).

However, other pot-culture experiments showed that N fertilizer significantly improved the biomass and Cd phytoremediation efficiency in *Tagetes patula* grown in Cd-contaminated soil (Ye et al., 2019). Nitrogen supply, particularly nitrate, sharply increased Cd extraction of *T. caerulea* (Schwartz et al., 2003), while urea significantly enhanced Cd phytoextraction by *Carpobrotus rossii* (Liu et al., 2016), which has further demonstrated that the addition of N fertilizer to soil plays an important role in Cd phyto-accumulation efficiency. Moreover, different types of N fertilizers exerted various effects on different plant species (Schwartz et al., 2003; Xie et al., 2009).

4.2 Effects of different fertilizers on H_2O_2 , MDA and proline contents and antioxidant enzyme activities in *S. nigrum* in relation to single and successive harvests

Cd-mediated stress can cause lipid peroxidation and subsequently produce large amounts of ROS, resulting in severe oxidative stress, that induces the production of low-molecular weight antioxidants, including proline and soluble proteins and certain antioxidant enzymes to protect against damages (Peng et al., 2015). It has been confirmed that N fertilizers effectively increase plant biomass by supplementing required nutrients, while enhancing the plant antioxidant system composed of enzymatic and non-enzymatic antioxidants. This in turn improves the resistance and tolerance of plants exposed to Cd stress (Jalloh et al., 2009), which is corresponding to another study on hyperaccumulator *Brassica juncea* grown in contaminated sediments, the research showed that N fertilizer not only increased boron phytoextraction, but also alleviates B stress (Giansoldati et al., 2012). Huang et al. reported that mineral fertilizers reduced the content of H_2O_2 and MDA in wheat (Huang et al. 2019). H_2O_2 and MDA are the major oxidation products of lipid peroxidation and can directly reflect the degree of oxidative stress. Accordingly, in our pot culture experiment, we discovered that after applying nitrogen fertilizer, the content of H_2O_2 and MDA in *S. nigrum* shoots at the maturation stage effectively decreased compared to CK ($P < 0.05$) (Table 3), which revealed that N fertilizers could remarkably strengthen the stress tolerance and promote the plant growth.

As one of the most commonly low-weight organic solutes, proline has a vital role in stabilizing protein complexes and scavenging free radicals on antioxidative system. It has been demonstrated that the endogenous proline overproduced in many plant species when subjected to abiotic and biotic stresses,

and the proline accumulation was closely related to H_2O_2 generation under stress condition (Ozfidan-Konakci et al., 2018). This is consistent with the results of our study that the H_2O_2 and proline concentration were considerably decreased after N treatment in *S. nigrum* shoots in different growth stages (Table 3 and Fig. 1 (a)). Moreover, some other studies showed that, in adversity circumstance, antioxidant defense mechanism were activated and antioxidant enzymes activities were enhanced, SOD combined with POD and CAT considered as the first defense barrier of the organisms, exerted a synergistic effect on ROS scavenging, therein, SOD converts $O_2^{\cdot-}$ to H_2O_2 and oxygen, whereafter, CAT and POD participate to scavenge H_2O_2 to O_2 to relieve the stress (Weydert et al., 2010). In our present study, we found that CAT activity was considerably decreased in *S. nigrum* shoots at the maturation stage, in contrast, POD as well as SOD activities increased (Fig. 1(b,c,d)). which was similar to the conclusions of Sun et al. (2015), who discovered that POD activity was elevated after fertilizer application (Sun et al., 2015). On the contrary, Huang et al. found decreased SOD activity after fertilizer application (Huang et al. 2019). The reason for this discrepancy is due to the change of antioxidant enzyme activities was correlated with many influence factors, such as plant species, fertilizer quantity and types, Cd concentrations and exposure times (Guo et al., 2019). Furthermore, SOD not only regulates the oxidative stress, but also plays an important role in the whole process of growth, development and reproduction in plants. In another study on two species of wheats, it is concluded that after the application of Si fertilizer, the antioxidant activity in Cd-tolerant cultivar remarkably improved than in Cd-sensitive cultivar (Naeem et al., 2018), which in-depth proved that antioxidant enzyme activities mainly depended on genotypic specificity.

In addition, we analyzed POD and SOD activities in *S. nigrum* shoots and they reached the maximum at the first and second florescence stages (Fig. 1(c,d)), which was consistent with the results of Esmaeili et al., who reported that antioxidant activity of *Oliveria decumbens* Vent. (Apiaceae) were the highest during flowering stages (Esmaeili et al., 2018).

4.3 Effects of different fertilizers on *S. nigrum* extractable Cd concentration in relation to single and double harvests

Our study showed that different types of N fertilizer supply were insufficient to significantly increase extractable Cd concentration compared to CK ($p < 0.05$) (Fig. 2). Generally, Cd availability and solubility affects extractable Cd concentration in the soil, and variation in Cd solubility depends on the pH value and organic matter content in soil (Ye et al., 2019).

4.4 Effects of different fertilizers on the pH value in comparison to single and double harvests of *S. nigrum*

Some articles have reported that after ammonium fertilizer soil application, the pH decrease in the soil might be attributed to NH_4^+ ion exchange and assimilation. However, in our study, after the application of N fertilizer, the pH value in the soil showed no significant change compared to CK without N fertilizer addition ($P < 0.05$) (Fig. 3). Nevertheless, only physicochemical conditions were taken into account from

the above conclusions, meanwhile, root exudates and metabolites released by growing plants and some microorganisms in the soil would regulate soil pH, so that plants could grow normally and healthily in the available root zone environment (Zhou and Song, 2004; Li et al., 2012).

5. Conclusions

Cd phytoremediation efficiency of *S. nigrum* improved with the addition of N fertilizers (NH_4HCO_3 , NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$, $\text{CH}_4\text{N}_2\text{O}$) ($P < 0.05$); moreover, Cd phytoaccumulation capacity of *S. nigrum* were higher during double harvests compared to single harvests due to the increase in *S. nigrum* shoot biomass and no obvious changes in Cd concentration. The pH value and extractable Cd content did not show a significant variation trend. In addition, analyzing the content and activity of non- and enzymatic antioxidants, we found that *S. nigrum* grew better after fertilization, and $(\text{NH}_4)_2\text{SO}_4$ treatment showed the highest efficiency in terms of Cd phytoremediation by *S. nigrum* harvested using a double-cropping system. However, more information is needed at the subcellular and molecular levels in order to gain deeper insight into the mechanisms.

Declarations

Authors Contributions Wei Yang: Resources, Data curation, Formal analysis, Writing - original draft; Huiping Dai: Data curation, Formal analysis, Methodology; Lidia Skuza Validation, Writing - review & editing; Shuhe Wei: Methodology, Funding acquisition, Project administration, Supervision

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Competing interests: The authors declare no competing interests.

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Tables

Table 1 Experimental treatment with 4 kinds of nitrogen fertilizers

No.	Treatment	Dose spiked (g kg ⁻¹)	Added total N (mg kg ⁻¹)	Harvest time
CK	Control, no N addition	0.00	0.00	at maturation stage
F1	NH ₄ HCO ₃	1.68	300.00	at the first florescence stage
F2	NH ₄ HCO ₃	1.68	300.00	at the second florescence stage
F3	NH ₄ HCO ₃	3.36	600.00	at maturation stage
F4	NH ₄ Cl	1.14	300.00	at the first florescence stage
F5	NH ₄ Cl	1.14	300.00	at the second florescence stage
F6	NH ₄ Cl	2.28	600.00	at maturation stage
F7	(NH ₄) ₂ SO ₄	1.41	300.00	at the first florescence stage
F8	(NH ₄) ₂ SO ₄	1.41	300.00	at the second florescence stage
F9	(NH ₄) ₂ SO ₄	2.82	600.00	at maturation stage
F10	CH ₄ N ₂ O	0.65	300.00	at the first florescence stage
F11	CH ₄ N ₂ O	0.65	300.00	at the second florescence stage
F12	CH ₄ N ₂ O	1.30	600.00	at maturation stage

Note: Doses of inorganic compounds are supplemented for analytically pure reagents

Table 2 Effects of different types of N fertilizers on Cd phytoextraction in *S. nigrum*

Treatment	Roots (mg kg ⁻¹)	Shoots (mg kg ⁻¹)	Shoot Cd extraction (µg plant ⁻¹)
CK	20.11±0.55a	20.61±0.63a	17.66±0.57e
F1	19.66±0.37a	20.38±0.31a	25.29±0.56d
F2	19.94±0.66a	21.05±0.26a	23.03±0.49d
F3	19.53±0.24a	21.08±0.25a	38.86±0.58b
F4	20.27±0.42a	21.00±0.45a	27.01±0.68d
F5	20.65±0.27a	21.05±0.23a	24.21±0.28d
F6	19.73±0.41a	20.27±0.34a	37.76±0.79b
F7	20.35±0.68a	21.09±0.21a	34.41±0.30c
F8	19.82±0.41a	21.00±0.30a	33.61±0.39c
F9	19.81±0.49a	21.07±0.05a	50.83±0.55a
F10	20.26±0.75a	21.35±0.32a	33.54±0.33c
F11	19.75±0.58a	20.80±0.23a	33.06±0.35c
F12	19.92±0.24a	20.84±0.48a	50.16±1.02a

Note: Means followed by the same letter within the same column are not significantly different (p<0.05)

Table 3 Effects of different types of N fertilizers on *S. nigrum* root and shoot biomasses (g plant⁻¹)

Treatment	Roots (g plant ⁻¹)	Shoots (g plant ⁻¹)
CK	0.16±0.01e	0.86±0.02e
F1	0.37±0.01cd	1.24±0.02d
F2	0.35±0.01d	1.09±0.01d
F3	0.41±0.01c	1.84±0.02b
F4	0.39±0.02c	1.29±0.01d
F5	0.37±0.02cd	1.15±0.01d
F6	0.42±0.01c	1.86±0.01b
F7	0.61±0.01b	1.63±0.03c
F8	0.57±0.01b	1.60±0.03c
F9	0.69±0.02a	2.41±0.01a
F10	0.61±0.01b	1.59±0.02c
F11	0.58±0.02b	1.59±0.02c
F12	0.70±0.01a	2.41±0.02a

Note: Means followed by the same letter within the same column are not significantly different (p<0.05).

Table 4 Effects of different types of N fertilizers on H₂O₂ and MDA in *S. nigrum* shoots

Treatment	H ₂ O ₂ (mg g ⁻¹ FW)	MDA (μmol g ⁻¹ FW)
CK	0.46±0.03a	6.27±0.04a
F1	0.31±0.02c	5.57±0.03c
F2	0.32±0.02c	5.55±0.03c
F3	0.39±0.03b	6.00±0.05b
F4	0.31±0.01c	5.51±0.02c
F5	0.30±0.02c	5.53±0.03c
F6	0.38±0.02b	6.02±0.07b
F7	0.33±0.03c	5.50±0.03c
F8	0.29±0.02c	5.52±0.04c
F9	0.37±0.02b	6.04±0.04b
F10	0.30±0.01c	5.49±0.04c
F11	0.34±0.02c	5.52±0.04c
F12	0.38±0.04b	6.03±0.05b

Note: Means followed by the same letter within the same column are not significantly different (p<0.05).

Figures

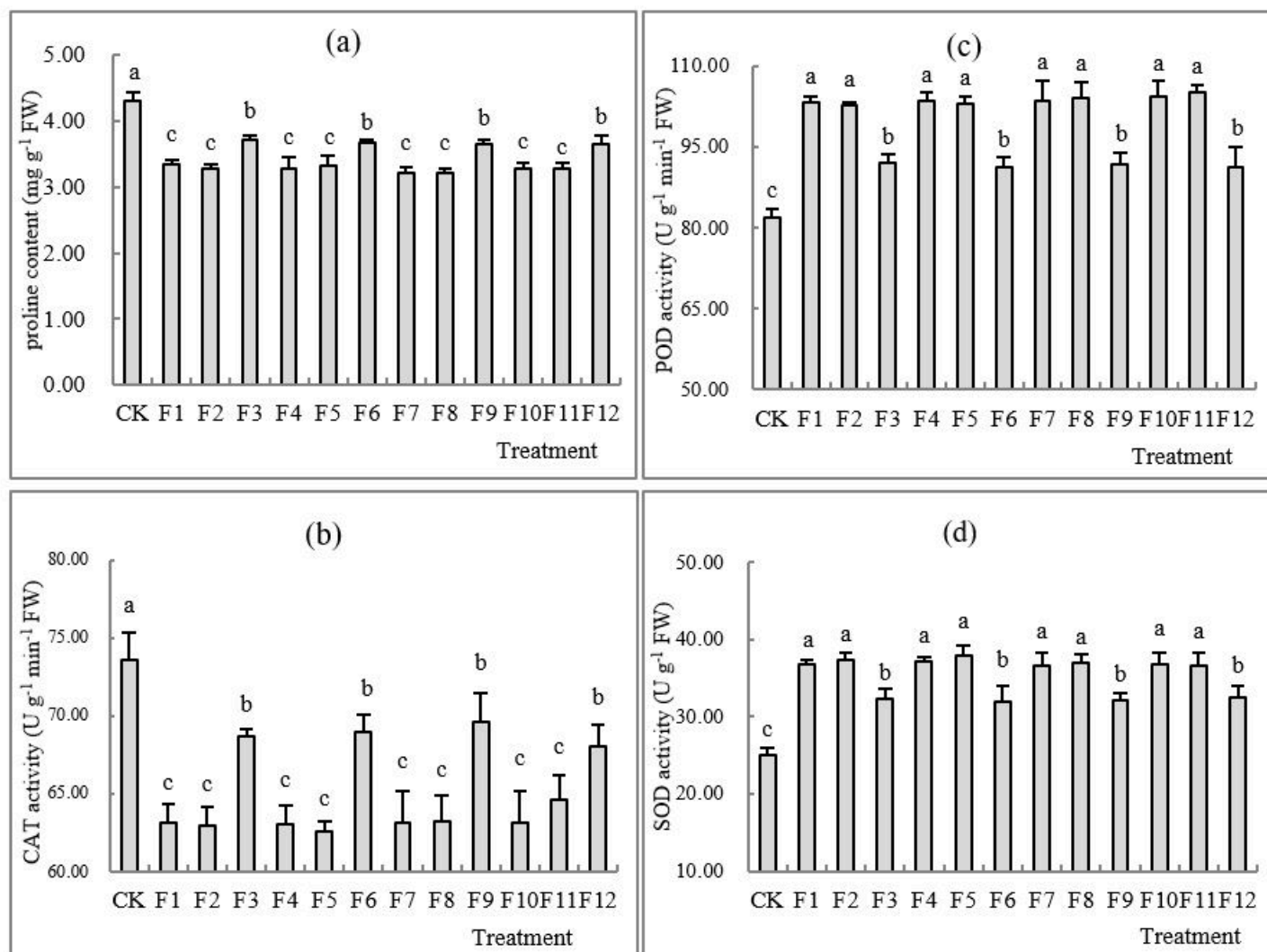


Figure 1

Effects of different types of N fertilizers on proline concentration (a) and the activity of CAT (b), POD (c) and SOD (d) by comparing single and double harvests in *S. nigrum* shoots (Means with different letters in the panel are significantly different among treatments at $P < 0.05$. Error bars reported in figures are means of 3 replicates with standard deviation)

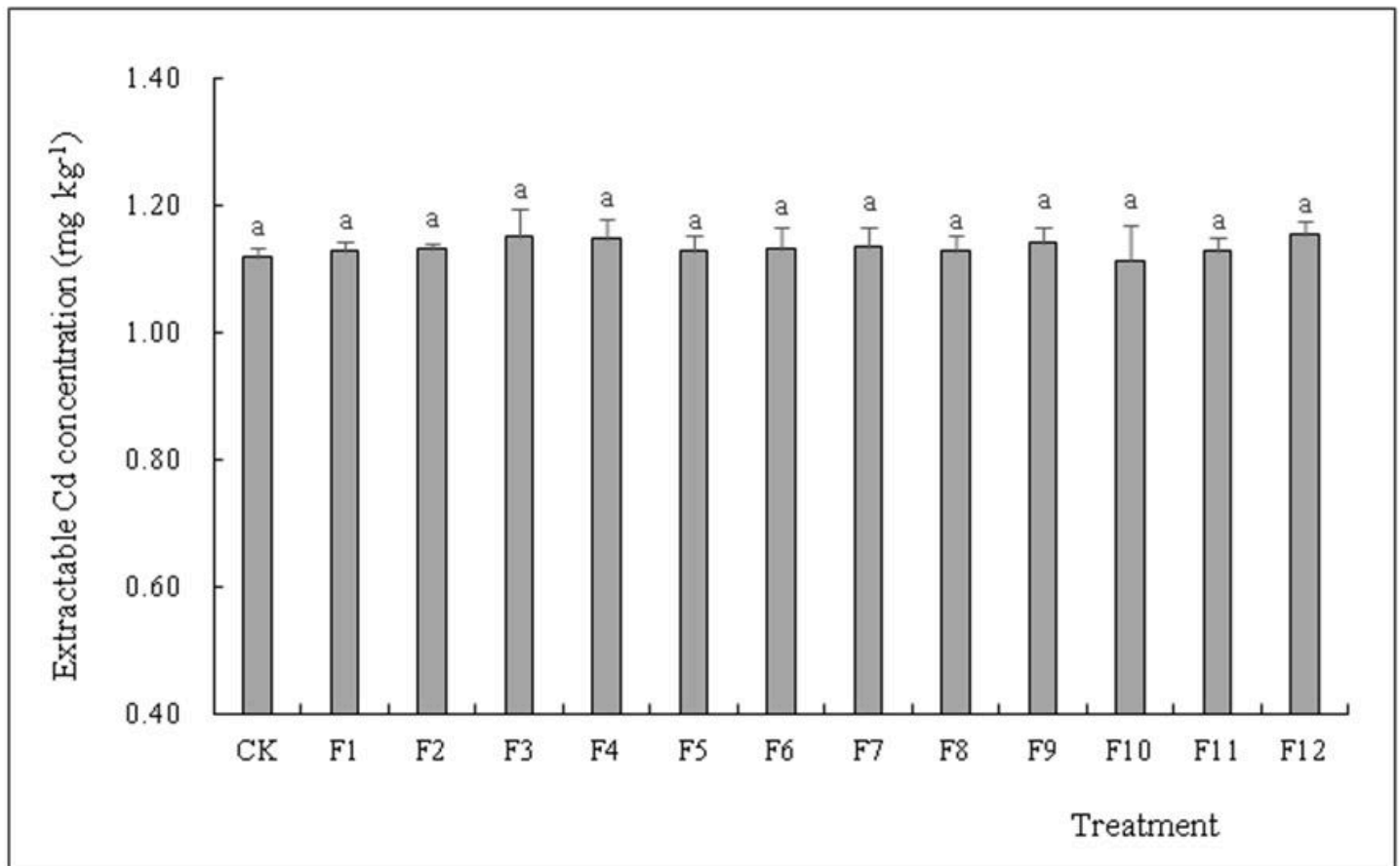


Figure 2

Effects of different types of N fertilizers on extractable Cd concentration by comparing single and double harvests in *S. nigrum* (Means with different letters in the panel are significantly different among treatments at $P < 0.05$. Error bars reported in figures are means of 3 replicates with standard deviation).

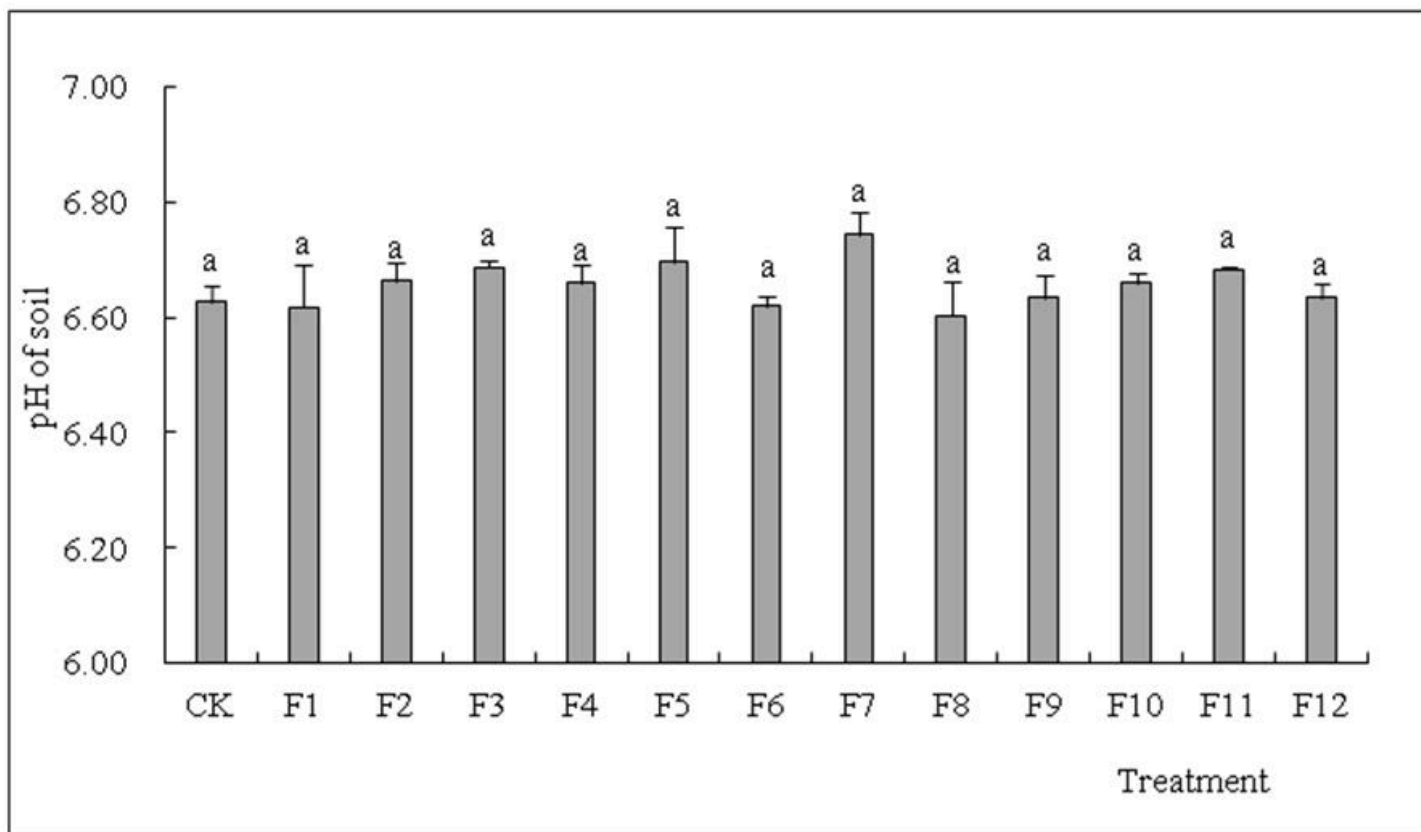


Figure 3

Effects of different types of N fertilizers on pH in soil by comparing single and double harvests in *S. nigrum* (Means with different letters in the panel are significantly different among treatments at $P < 0.05$. Error bars reported in figures are means of 3 replicates with standard deviation).