CHEMICAL ENGINEERING TRANSACTIONS
VOL. 62, 2017
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Fei Song, Haibo Wang, Fang He

**ISBN** 978-88-95608- 60-0; **ISSN** 2283-9216
DOI: 10.3303/CET1762217

# Study on Mitigative Effects of Exogenous No Donor Sodium Nitroprusside on Corn Nickel Toxicity

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Ttested material are corn and sodium nitroprusside. Cultivation and experimental analysis are used to research influence of nickel, sodium nitroprusside and its different concentration ratio on germination of corn seeds. The observation objects include germination percentage of seeds, germination potential, germination index and vitality index; meanwhile, this paper also researches the mitigative mechanism of sodium nitroprusside on growth of corn seedlings and nickel toxicity under nickel stress, and selects the overground and underground growth and physiology and biochemistry indexes etc. as the observation objects. Through analysis of relevant data, following results are obtained: nickel concentration test proves that different nickel treatment concentration has different influence on germination of corn seeds. Nickel with low concentration can slightly promote germination of corn seeds and nickel with high concentration suppresses the corn seeds. Sodium nitroprusside experiment proves that sodium nitroprusside with low concentration can promote the germination of corn seeds and sodium nitroprusside with high concentration has a suppression effect.

## 1. Introduction

Contents of heavy metals in the soil is increasing along with industrial and agricultural development and human activities and utilization of pesticides and chemical fertilizers (Aghaei-Amirkhizi et al., 2016). It's estimated that the amount of heavy metals entering the soil in various ways per year in the world is about tens of millions of tons and is increased year by year. According to statistical results in 1998, agricultural acreage polluted by heavy metals in China accounts for 30%-40% of all agricultural acreage polluted in any way, so as to cause that China suffers from losses of more than ten million tons of cereals per year (Batool et al., 2014). Up to 12 million tons of cereals are not available due to being polluted by heavy metals, so as to leave great influence on people's life. Nickel is one of multiple heavy metal pollutants. It is difficult to be degraded after entering the soil and continuously enriched inside the organism through food chain (Cao et al., 2014). And, research also finds that nickel is characterized in potentiality, concealment, diversity, wide range of pollution and long duration in the environment (Chen et al., 2017). So, deep research of nickel plays an important practical significance in guiding agricultural production and healthy life. At present, more and more attention has been paid to the study of nickel pollution at home and abroad (Chen et al., 2017).
Northeast region of China plays a very important role in the national economic development. Corn is a food crop widely planted in this area and an important grain, feedstuff and industrial raw materials. Its yield and quality are closely linked to national economy and the living standards (How et al., 2013). But, in recent years, nickel content in the black soil, main producing area of corn the northeast, is increasing continuously (Lin et al., 2017). In some areas, nickel pollution has been found (Ma et al., 2012), which worsens the physical and chemical properties of the black soil and reduces the yield and quality of corns. There, study on mitigative effects of exogenous NO on corn nickel toxicity plays an important practical significance in preventing nickel pollution, improving crop yield, ensure crop quality and prevent the harm of nickel (Mansour et al., 2016).

## 2. Materials and methods

Variety of test corns is "Ji Nong 669" (Jilin Agricultural University); nickel is nickel sulphate (purchased from Sinopharm Chemical Reagent, analytically pure); NO donor is sodium nitroprusside (SNP, purchased from

Please cite this article as: Yangwu Liu, Ping Luo, 2017, Study on mitigative effects of exogenous no donor sodium nitroprusside on corn nickel toxicity, Chemical Engineering Transactions, 62, 1297-1302 DOI:10.3303/CET1762217

Sinopharm Chemical Reagent, analytically pure), compound when it is in need, NO production rate of SNP with concentration 0.15 mmol/L should be 0.2μmol/L.

### 2.1 Experimental design

Several full seeds of uniform size, petri dish, sterilized for 10 minutes by using 10% H2O2, and flushed repeatedly by distilled water before test:
Influence of nickel with different concentrations on germination index of corn seeds.
In the test, the set concentration of the nickel is respectively 0mmol/L, 0.1 mmollL, 0.5 mmol/L, 1 mmol/L and 2 mmol/L. Treated corn seeds are submerged in the nickel solution with different concentration for three times. There are 15 petri dishes in total. Each petri dish is provided with 50 seeds for cultivation and nickel solution is changed once a day.
Mitigative effects of different sodium nitroprusside on corn germination under nickel stress. Sodium nitroprusside solutions (SNP) with different concentrations (0 mmol/L, 0.05 mmol/L, 0.1 mmol/L are respectively expressed by SNPO, SNP0.05 and SNP0.1) are used for soaking the seeds. After 24h, distilled water are used for flushing repeatedly. Then, treated seeds are respectively submerged in the nickel solutions with different concentrations (0 mmol/L, 0.5 mmol/L and 1.0 mmol/L are respectively expressed by Ni0, Ni0.5, and Ni1.0) for three repeated times. There are 27 petri dishes in total. Each petri dish is evenly provided with 50 seeds and nickel solution is changed once a day.

### 2.2 Determination method

Extraction of enzyme solution: taking 0.5g leaf and adding with 1.5ml phosphate buffer (0.05 mol /L, pH=7.8). After ice-bathing and grinding, 1.5ml phosphate buffer is poured into the centrifugal tube. 2 ml buffer solution are used for cleaning the bowl, which is placed at (0-4°C) for centrifuging for 20min at 4000 rpm. Then, the supernatant is crude enzyme extraction, which can be directly used for determining activity of SOD (superoxide dismutase), POD (peroxidise) and CAT (catalase) and content of MDA (malonaldehyde).
Determining activity of superoxide dismutase (SOD): 3ml reaction solutions (including 2.7 ml 144 mmol/L methionine, 0.1 ml 22.5 μmol/L nitrogen tetrazole, 0.1 ml 3 μmol/L EDTA-Na and 0.1 ml 60 μmol/Lriboflavin) are added to 2ul enzyme extracting solution. Then, it reacts for 30min under illumination of 4000 lux. 560nm colorimetric is used to suppress photochemical reduction of NBT and 50% of them should be one enzymatic activity unit.

## 3. Experimental design and results

Through test on germination of corn seed with indoor incubation at constant temperature, it's necessary to firstly determine the germination rate, germination potential, germination index and vitality index of corn seeds at different nickel concentrations, further measure the germination rate, germination potential, germination index and vitality index of corn seeds at different sodium nitroprusside concentrations, and confirm the selection of concentration of nickel sodium nitroprusside. On this basis, it also determines the mitigative effect of sodium nitroprusside under nickel stress on germination of corn seeds. All above results are used to simplify the part. Sodium nitroprusside is 0.00 mmol/L, 0.05 mmol/L and 0.10 mmol/L in concentrations. When the corn grows two leaves and one center, 0.0 mmol/L and 1.0 mmol/L are used for treatment.

### 3.1 Influence of nickel treatment with different concentrations on germination of corn seeds

During measurement of germination percentage of corn seeds and other parameters, it also measures the root length, bud length and its total fresh weight after germination of corn seeds at nickel concentration 0mmol/L, 0.1mmol/L, 0.5 mmol/L, 1 mmol/L and 2 mmol/L. Research results are shown in Table 1. It can be seen from Table 1 that with increasing concentration of the nickel, root length of the corn is increased greatly and later slowly (Wang et al., 2015).
Different concentrations of the nickel have different influence on germination of corn seeds. Low-concentration nickel can slightly promote the germination of corn seeds (Park 2015) and high-concentration nickel may suppress the corn seeds. The suppression effect of nickel on corn bud is less than on root (Sharma et al, 2013). Through comparison of suppression effect of nickel at different concentrations on germination of corn seeds, 0.5mmo1/L and 1.0mmol/L are used as the stress concentration of the nickel (Shimokawa et al., 2012).

### 3.2 Mitigation of sodium nitroprusside treatment on corn germination rate under nickel stress

After cultivation for 72 hours, it's necessary to determine germination percentage of treated seeds, and the results are shown in Figure 1: if used nickel amount is 0.00 mmo1/L, sodium nitroprusside at different concentrations improves the germination rate of corn seeds in different degrees, which has been mentioned above; if concentration of nickel stress is 0.5mmol/L, compared with the blank, nickel at this concentration

obviously reduces the germination rate of corn seeds. But, 0.05mmol/L and 0.10mmol/L sodium nitroprussides are obviously used to improve the germination rate of corn seeds. Compared with single 0.5mmol/L nickel treatment, there is a significant difference level. Compared with blank treatment, 0.05mmol/L sodium nitroprusside treatment is implemented to achieve a significant difference level. But, 0.10mmol/L sodium nitroprusside treatment isn't implemented to achieve a significant difference level, it means that through treatment by 0.10mmol/L sodium nitroprusside, it can effective mitigate the influence of 0.5mmol/L nickel concentration on germination rate of the corn, which can be basically restored to the control level (Sitorus H. B. H. et al, 2015).

*Table 1: The Influence of Different Nickel Treatment on Corn Seed Root Length, Shoot Length and Fresh Weight*

| Treatment | Nickel concent (mmol/L) | Root length(cm) | General length(cm) | Fresh weight(mg/) |
|---|---|---|---|---|
| 1 | 0.0 | 3.48±0.22bB | 2.21±0.05bB | 11.40±1.01bB |
| 2 | 0.1 | 4.11±0.10aA | 2.46±0.10aA | 19.05±0.72aA |
| 3 | 0.5 | 3.34±0.15bB | 2.16±0.06bB | 11.04±0.21bB |
| 4 | 1.0 | 2.46±0.12cC | 1.84±0.02cB | 8.60±0.63cC |
| 5 | 2.0 | 1.67±0.09dD | 1.47±0.11dC | 4.8511.04±0.21bB0.39dD |

(Note: if the lowercase in the table refers to significance level of difference, the capital letter should refer to extreme significance level of difference; degree of freedom for processing and error should be and 10, respectively).

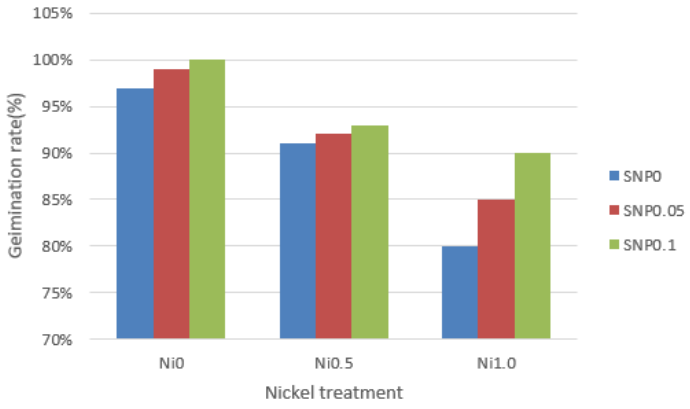


*Figure 1: The influence of SNP treatment on corn seed germination rate under nickel stress*

### 3.3 Mitigation of sodium nitroprusside treatment on corn germination potential under nickel stress

During measurement of several pairs of corn germination rate, it also measures the germination potential of the corn. The results are shown in Figure 2: it can be seen from the diagram that when used nickel amount is 0.0mmol/L, sodium nitroprusside at different concentration can improve the germination potential of corn seeds in a different degree; when concentration of nickel stress is 0.5mmol/L, compared with the blank, the nickel at this concentration is obviously used to reduce the germination potential of corn seeds and 0.05mmol/L and 0.10mmol/L sodium nitroprusside are used to obviously improve germination potential of corn seeds. The increased range compared with single nickel is 2.3% and 5.6% respectively, which have reached the significant difference level. Compared with the blank, 0.10mmol/L sodium nitroprusside treatment isn't used to achieve the significant difference level, which means that 0.10mmol/L sodium nitroprusside can be used to mitigate the influence of 0.5mmol/L nickel on germination potential of corn, which can be basically recovered to the control level (Zhang et al., 2015).

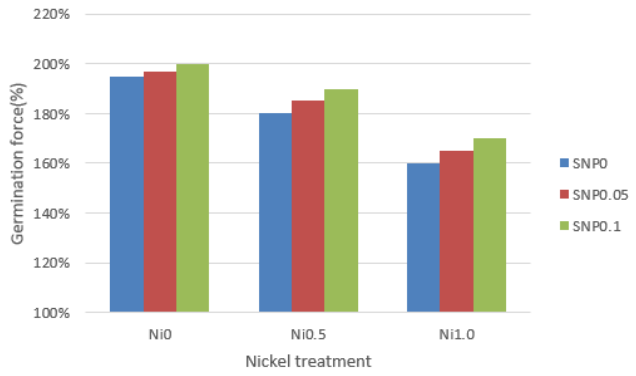


*Figure 2: The Influence of SNP Treatment on Corn Seed Germination Force under Nickel Stress*

### 3.4 Mitigation of sodium nitroprusside treatment on corn germination index under nickel stress

Similarly, it's also necessary to study the mitigation of sodium nitroprusside treatment on corn germination index under nickel stress. Study results are shown in Figure 3. It can be seen from the figure that when nickel stress concentration is 0.5mmol/L, compared with the blank, nickel at this concentration is obviously used to reduce germination index of corn seeds. But, 0.05mmol/L and 0.10mmol/L sodium nitroprusside treatment are obviously used to improve germination index of corn seeds. Compared with single nickel treatment, the increased range is up to 0.9% and 1.8% respectively without reaching the significant difference level. Compared with the blank, 0.10mmol/L sodium nitroprusside treatment is also not up to the significant difference level, it means that 0.10mmol/L sodium nitroprusside treatment has certain limitation for mitigating corn germination index at this concentration. Specific reasons still need further study.

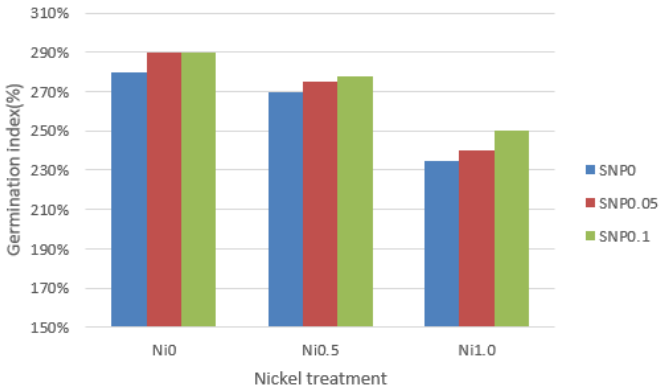


*Figure 3: The influence of SNP treatment on corn seed germination index under nickel stress*

### 3.5 Influence of sodium nitroprusside treatment on dry and fresh weight of corn under nickel stress

In order to study mitigation of different sodium nitroprusside concentrations on corn seedlings, it's necessary to measure the fresh and dry weight of corn seedlings under different treatment conditions. Study results are shown in Figure 4 and Figure 5.

It can be known from the figure that compared with NiO+SNPO treatment, NiO+SNP0.05 treatment and N10+SNP0.1 are used to significantly improve fresh weight of corn seedlings for 14.6% and 30.5%, respectively. Dry weight of corn seedlings is slightly increased without reaching the significant difference level. It means that two sodium nitroprusside concentrations under the test can be used to significantly improve the fresh weight of corn seedlings, which has no significant influence on dry weight of corn seedlings.

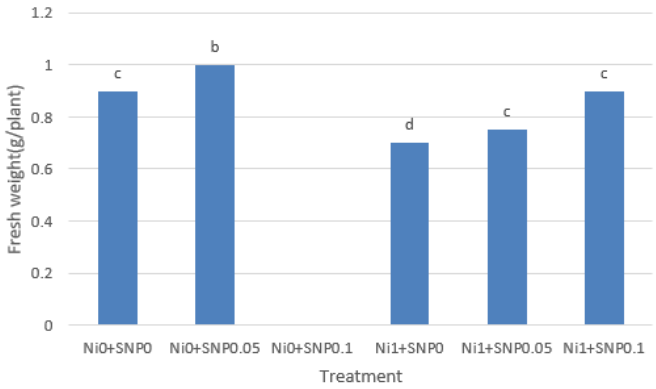


*Figure 4: Effects of SNP treatment on the corn seedling fresh weight under nickel stress*

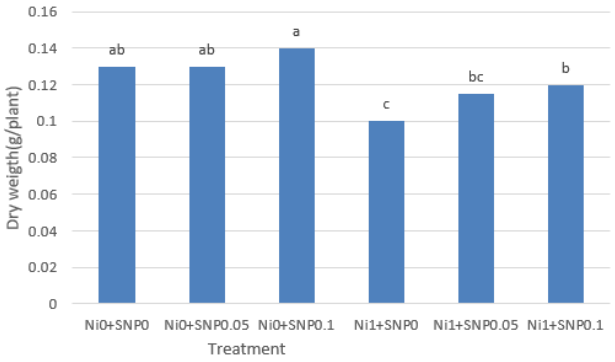


*Figure 5: Effects of SNP treatment on the corn seedling dry weight under nickel stress*

## 4. Conclusions

In order to testify that different nickel concentration has a suppression effect on germination of corn seeds, 0.5mmol/L and 1.0mmol/L are used as nickel stress concentration. Experiment proves that different nickel concentration has different influence on germination of corn seeds. Nickel with low concentration may slightly promote the germination of corn seeds and high concentration has a suppression effect. Suppression of nickel for buds is less than those for roots. In order to prove that different concentrations of sodium nitroprusside can improve the germination of corn seeds, 0.05mmol/L and 0.10mmol/L are used to mitigate the nickel stress. Experiment proves that sodium nitroprusside with different concentrations have influence on germination of corn seeds. Low concentration can promote the germination of corn seeds and high concentration has a suppression effect. Sodium nitroprusside has a larger effect in promoting corn buds than roots.

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