VOL. 6, NO. 9, SEPTEMBER 2011 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science

www.arpnjournals.com

EFFECT OF CADMIUM, LEAD AND NICKEL SALT SOLUTIONS ON THE GERMINATION AND EARLY GROWTH OF SPINACH (*Spinacia oleracea* L.)

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ABSTRACT

The effects of lead, cadmium and nickel on the germination and early growth of spinach seedlings were investigated. The laboratory experiments were carried out in Petri dishes that contained 1g of cotton wool as sowing medium under photo and non-photo periodic conditions. The results of the study showed the coefficient of germination velocity of the spinach seeds decreased with increase in concentrations of the metal solutions in the media. The results also showed that lead, cadmium and nickel did not inhibit germination of spinach seeds but impaired the growth and seedling establishment. The spinach seedlings tolerated more of lead than either cadmium or nickel. The highest concentration of each of the metals that did not support seedling growth is in the sequence nickel < cadmium < lead.

Keywords: spinach, cadmium, lead, nickel, germination, early growth.

INTRODUCTION

Spinach is an excellent source of vitamins, K, A, C, E, B2, B6, folate and trace minerals such as manganese, zinc, copper, selenium, magnesium, iron, calcium and potassium. It is a very good source of omega-3 fatty acids, niacin, protein, and dietary fiber (Wiki, 2008).

Trace metal contamination of spinach commonly arises from pollution of agricultural farmlands. Sources of pollution of agricultural farmlands by trace metals in the natural terrestrial ecosystem include air-borne metals from automobile exhaust fume and industrial activities (Audu and Lawal, 2005; Jaja and Odoemena, 2004; Odoemena, 1999; Shapek, 1996). Farmers often use industrial effluents (Subramani *et al*., 1998) as a source of irrigation when there is a shortage of water (Jamal *et al*., 2006; Saleemi, 1993). In addition to the soil contamination, a significant toxic chemical may be taken up by the crop and carried to the food chain (Liphadzi and Kirkham, (2006); Pescod and Alka, 1985). Trace metal phytotoxicity is considered to be the main factor limiting plant growth and crop cultivation especially on acid soils (Jamal *et al*., 2006; Foy *et al*., 1978). Lead acetate and copper salts have been reported to have high inhibitory tendencies on the germination and growth of tomato varieties (Jaja and Odoemena, 2004). A green house experiment has also confirmed the phytotoxic effect of aluminium and chromium on the germination and early growth of wheat varieties (Jamal *et al*., 2006). Localizations of aluminum in root tips of *Zea mays* and *Vicia faba* has also been reported (Marienfield *et al*., 2000). Interactive effects of supplemental ultraviolet-B radiation and heavy metals on the growth and biochemical characteristics of *Spinacia oleracea* in pot soils have been reported (Shweta and Agrawal, 2006). According to this study, the interactive effects of both stresses were less than additive, but combined treatment of ultra violet-B radiation (UV-B) and cadmium was more deleterious than the UV-B and nickel combination. Operationally defined fractions of cadmium that control the availability of cadmium to spinach correlated with dry-matter yield of spinach crop in pot soil experiments (Adhikari *et al*., 2005).

The objective of the present study is to determine the effects of lead, cadmium and nickel on the germination and early growth of *Spinacia oleracea* in Petri dishes in order to preclude soil factors (Richard, 2008) in soil pot experiments. Lead, nickel and cadmium have been selected for this study because of their widespread occurrence and potential for their toxicities (Smith, and Flegal, 1995).

MATERIALS AND METHODS

Semi Savoy spinach seeds were obtained from vegetable market in Kaduna central market, Nigeria. The seeds were surface sterilized with 1.2% sodium hypochloride solution for 30 seconds and rinsed several times with deionised water. Sterilized Petri dishes of approximately 9cm in diameter each containing 1g of neatly spread cotton wool were used as sowing container and media. Lead was applied as lead acetate, cadmium was applied as cadmium chloride and nickel was applied as nickel ammonium sulphate. The concentrations of lead, cadmium and nickel selected for experiments fall within the normal levels recommended for metals in plants, as reported by Awofolu *et al*., (2005). Contamination of the media was established by adding 5, 6, 7, 8, 9 and 10mg lead in each case in $25cm^3$ deionised water which was found adequate for moistening during the period of study. Contamination with cadmium was established by adding 0.1, 0.5, 1.0, 1.5, 2.0 and 2.5 mg cadmium in each case, in $25cm^3$ deionised water. Contamination with nickel was similarly established by adding 0.1, 0.5, 1.0, 2.0, 3.0 and 4.0 mg nickel in each case in $25cm^3$ deionised water.

VOL. 6, NO. 9, SEPTEMBER 2011 ISSN 1990-6145
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Twenty seeds of spinach were sown in each Petri dish for different metal treatment levels and observed for germination. Control seed germination experiments were similarly carried out in Petri dishes containing neatly spread cotton wool, soaked in only 25cm^3 deionised water without any of the metals studied. The criterion for seed germination was the emergence of 2mm radicle at the time of observation (Odoemena, 1988). Germination counts were recorded at 12 and 6 hours interval for 72 hours, after sowing. The seedling growth and development response was observed until the plant turned yellowish and died. The germination percentage of seeds was determined for each treatment and the data were expressed as Aggregate Germination Percentage (AGP) and Coefficient of Germination Velocity (CGV). Hartmann and Kester, (1964) expressed CGV as follows.

$$CGV = \frac{\text{Total number of seedling}}{A_1T_1 + A_2T_2 + A_xT_x}$$

Where A = number of seedlings emerging on a particular number of days (T) and subscripts 1, 2.........x are respective number of germinated seeds per respective number of days after sowing of the seeds. The experiment was terminated after 90 hours.

RESULTS

The number of seedlings with 2mm radicle emerging per day is shown in Tables 1, 2 and 3 for different levels of lead, cadmium and nickel treatments, respectively.

Table-1. The number of seedlings with 2mm radicle emerging per day for different levels of lead treatment.

Amount of lead (mg)	**Number of seedlings emerging per day**								
	Days								
	1/2	**3/4**	**1**	**1 1/2**	**1 3/4**	**2**	**2 1/2**	**2 3/4**	**Total seedlings**
0	0	0	10	6	3	0	0	0	19
5	0	0	3	8	2	5	0	0	18
6	0	0	1	11	0	3	1	2	18
7	0	0	2	10	1	0	2	5	20
8	0	0	0	4	5	2	4	1	16
9	0	0	0	5	2	3	5	5	20
10	0	0	0	2	2	3	8	3	18

Table-2. The number of seedlings with 2mm radicle emerging per day for different levels of cadmium treatment.

Amount of cadmium (mg)	**Number of seedlings emerging per day**								
	Days								
	1/2	**3/4**	**1**	**1 1/2**	**1 3/4**	**2**	**2 1/2**	**2 3/4**	**Total seedlings**
0	0	4	7	7	0	0	0	0	18
0.1	0	4	11	4	0	0	0	0	19
0.5	0	3	6	9	0	0	0	0	18
1.0	0	2	9	9	0	0	0	0	20
1.5	0	2	7	8	0	2	0	0	19
2.0	0	0	3	12	0	2	0	0	17
2.5	0	0	3	10	0	3	0	0	16

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Table-3. The number of seedlings with 2mm radicle emerging per day for different levels of nickel treatment.

Amount of nickel (mg)	Number of seedlings emerging per day								
	Days								
	1/2	3/4	1	1 1/2	1 3/4	2	2 1/2	2 3/4	Total seedlings
0	0	0	5	12	1	0	0	0	18
0.1	0	0	7	10	1	1	0	0	19
0.5	0	0	5	10	1	2	0	0	18
1.0	0	0	2	14	0	3	0	0	19
2.0	0	0	0	12	3	4	0	0	19
3.0	0	0	0	12	3	2	1	0	18
4.0	0	0	0	12	2	3	1	0	18

From the results in Tables 1, 2 and 3, the spinach seeds germinated well under all the different levels of the metal treatments. Aggregate Germination Percentage (AGP) and the Coefficient of Germination Velocity (CGV) of spinach seeds sown in lead, cadmium and nickel separately contaminated media are presented in Tables 4, 5 and 6, respectively.

Table-4. Aggregate germination percentage (AGP) and the coefficient of germination velocity (CGV) of spinach seeds sown in lead contaminated media.

Amount of lead (mg)	AGP (%)	CGV
0	95	0.784
5	90	0.632
6	90	0.576
7	100	0.544
8	80	0.496
9	100	0.462
10	90	0.442

Table-5. Aggregate germination percentage (AGP) and coefficient of germination velocity (CGV) of spinach seeds sown in cadmium contaminated media.

Amount of cadmium (mg)	AGP (%)	CGV
0	90	0.878
0.1	95	0.950
0.5	90	0.828
1.0	100	0.833
1.5	95	0.776
2.0	85	0.520
2.5	80	0.667

Table-6. Aggregate germination percentage (AGP) and the coefficient of germination velocity (CGV) of spinach seeds sown in nickel contaminated media.

Amount of nickel (mg)	AGP (%)	CGV
0	90	0.727
0.1	95	0.738
0.5	90	0.699
1.0	95	0.655
2.0	95	0.608
3.0	90	0.605
4.0	90	0.600

The coefficient of germination velocity of spinach seeds generally decreased with increase in levels of lead and nickel treatments. However, the germination velocity of spinach seeds in media contaminated with cadmium did not show this trend.

The number of seedlings that survived and grew above the brim of the Petri dish after 90 hours was the growth determinant. The numbers of seedlings that survived and grew above the brim of the Petri dishes within the experimental period with lead, cadmium and nickel contaminations are shown in Tables 7, 8 and 9 for lead, cadmium and nickel, respectively.

The linear dimension growth study indicates that increased levels of metal contaminants reduced the number of seedlings that grew above the brim of the Petri dishes.

Table-7. Number of seedlings that survived and grew above the brim of the Petri dishes with lead contamination.

Amount of lead (mg)	**Number of seedlings that grew above Petri dish brim**
0	18
5	8
6	5
7	7
8	2
9	3
10	0

Table-8. Number of seedlings that survived and grew above the brim of the Petri dishes with cadmium contamination.

Amount of cadmium (mg)	**Number of seedlings that grew above dish brim**
0	16
0.1	14
0.5	4
1.0	2
1.5	0
2.0	0
2.5	0

Table-9. Number of seedlings that survived and grew above the brim of the Petri dishes with nickel contamination.

Amount of nickel (mg)	**Number of seedlings that grew above dish brim**
0	18
0.1	16
0.5	1
1.0	0
2.0	0
3.0	0
4.0	0

DISCUSSIONS

The various levels of lead, cadmium, and nickel used in the germination and growth study did not substantially prevent seed germination (Tables 1, 2, 3). Metals such as chromium and aluminium have also been reported not to inhibit germination but impair growth and seedling establishment (Rellen-Alvarez *et al*., 2006). Spinach seed germination in lead treated media, presents the least aggregate germination percentage (AGP) of 80% which was recorded in the Petri dish treated with 8mg/25cm^3 lead solution. The Petri dishes treated with higher concentrations of 9 and 10 mg/25cm^3 recorded 100 and 90% germination, respectively (Tables 1, 4). The lack of trend in these results may be associated with the presence of dormant seeds among the seeds planted. Similar observations were made on media contaminated with cadmium and nickel salt solutions. According to Jaja and Odoemena, (2004), increase in metal concentration may increase suppression of seed germination. Suppression of seed germination and plant growth responses have been attributed to the establishment of higher toxic effect syndrome due to high accumulation of the metals within the plant body biomass (Singh and singh, 1981; Esenowo, 1995)

In the present study, suppression of the germination of the spinach seeds manifested in delay in germination with increase in concentrations of lead, cadmium, and nickel salts. This observation explains why the coefficients of germination velocity (CGV) decreased as the concentrations of the metal salts were increased in the germination media (Tables 4, 5, 6). The decreases in the values of AGP and CGV of the spinach seedlings caused by the increased metal levels indicate that at lower concentrations of the metal contaminants, they posed little or no harm on the seeds viability but at higher concentrations, germination and growth may be retarded. This is consistent with earlier reports by Jaja and Odoemena, (2004). Cadmium and nickel germination media treatments at the concentration level of 0.lmg/25cm^3 for each medium were observed to stimulate the germination rate of the spinach seeds. This is evident from the lower CGV and AGP values for their respective controls and higher number of seedlings that grew above the brim of the Petri dishes for cadmium and nickel contaminated media (Tables 8, 9). The highest concentrations of cadmium and nickel that did not support seedling growth were 1.5 and 1mg/25cm^3 solution respectively. Lead presented an astonishing trend of supporting seedling growth up to a concentration of 9mg/25cm^3. However, only few spinach seedlings (15-40%) enjoyed this support. Abdullahi *et al*., (2008) reported that nickel is a naturally occurring element that is found in enzyme urease and is considered to be essential for plant growth. The ability of low concentrations of metals to stimulate plant growth was also reported by Jamal *et al*., (2006) and Jaja and Odoemena, (2004). The shoot growth response decreased with increase in metal salt contents in the growing media. This linear dimension growth study indicates that increased levels of metal contaminants produced spinach growth inhibition to the extent of seedling mortality. Adhikari *et al*., (2005) also reported that above 80mg Cd per kilogram soil, there is very slow growth of spinach after germination.

Most of the spinach seedlings turned yellow at three days, before they died. The yellow coloration is attributable to chlorosis (Bernstein, 1961). Shweta and Angrawal, (2006) reported that the characteristic feature of toxicities in plants due to heavy metal is chlorosis and reduction in net photosynthetic rate which leads to decrease in plant growth and productivity.

CONCLUSIONS

The study showed that lead, cadmium and nickel did not inhibit germination of spinach seeds but impaired the growth and seedling establishment. The spinach seedlings tolerated more of lead than either cadmium or nickel. The highest concentration of each of the metals that did not support seedling growth is in the sequence nickel < cadmium < lead.

ACKNOWLEDGEMENTS

We acknowledge the assistance of the laboratory staff of Department of Applied Science, Kaduna Polytechnic.

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