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Establishing Critical Limits for Nickel in Soil and Plant for Predicting the Response of Spinach (*Spinacia oleracea*)

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Nickel (Ni) is an essential element for plants and research reports are available indicating its beneficial effects on growth of higher plants as well. Though abundant information exists on Ni toxicity in soil and plant system but not much is available on its critical level of deficiency (CLD) in soils and plants. A pot experiment was conducted in net-house of the micronutrient research project, Anand Agricultural University, Anand, Gujarat. For assessing the critical limit in soil, three bulk soils *viz.*, low (<0.5 mg Ni kg^{-1}), medium (0.5 to 1.0 mg Ni kg^{-1}) and high (>1.0 mg Ni kg^{-1}) were collected from different locations of Anand district. Six levels of Ni, *i.e.* Ni_0, Ni_2, Ni_4, Ni_6, Ni_8 and Ni_{10} (0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg Ni kg^{-1} soil) were applied in all 20 soils (10 low, 6 medium and 4 high in Ni content in soil). The experiment was conducted in factorial completely randomized design with three replications. The critical limit of Ni in soil was determined by Bray's per cent yield plotted against soil available Ni using the scattered diagram in graphical as well as statistical method. Wide variation in dry matter produce was observed across the soils. The concentration of Ni in spinach plant increased with increasing the level of Ni in soil. The CLD of the 0.005 *M* DTPA-$CaCl_2$ extractable Ni in soil and plant was worked out as 0.46 and 2.27 mg kg^{-1}, respectively with statistical method. Whereas, in graphical method it was reported as 0.50 and 2.20 mg kg^{-1}, respectively in spinach crops.

Key words: Critical limit, nickel application, spinach, crop response

Nickel (Ni) is relatively abundant and naturally occurring metal, widely distributed in the earth's crust. On an average, Ni concentration in earth's crust is about 50 mg kg^{-1} (Alloway 1990). The deficiency of Ni is more likely to be increased in Indian soils as improved agronomic practices favour rapid growth of crops on soils prone to low Ni content (Katyal and Rattan 1995).

Nickel is essential for plants but the concentration in majority of plants species ranged between 0.05 to 10 mg kg^{-1} of the dry matter (Pande *et al.* 2012; Singh and Mishra 2012). Nickel content in soils varied widely and has been estimated to be ranged from 3 to 1000 mg kg^{-1}; for the world soils, the range is between 0.2 to 450 mg kg^{-1}, while the grand mean is calculated to be 22 mg kg^{-1} (Cempel and Nikel 2006). Solubility and mobility of Ni increases with decreasing pH, therefore soil pH is the major factor for controlling Ni solubility as well as mobility in the soils (Tye *et al.* 2004). The discovery that Ni is a component of the plant urease in 1975 (Dixon *et al.* 1975) prompted a renewed interest in the role of Ni in many plants even for higher plants like legumes (Marschner 2003). Critical limit for a crop under well-defined condition involves comparison of concentration of nutrient in the plant known to be deficient with that supplied adequately with the given nutrient. Thus, the response of crop plants to the insufficiency or sufficiency of specific nutrients has helped to generate information on the critical limits of each of the elements (Bates 1971). Barman *et al.* (2020) evaluated the crop response of applied for assessing the available Ni in soil using soybean as test crop and found 16.5 to 26.6 per cent increase in the biomass yield of soybean to applied Ni (5 mg kg^{-1}) over control. It has also been reported that growth and yield attributes of barley increased up to the application of 10 mg Ni kg^{-1} soil whereas concentration and uptake of Ni increased with increasing levels of Ni supply (Kumar *et al.* 2018b). The critical limits or levels is quite often employed

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for a wide variety of soils and crops, even though these critical limits may be different not only for soils and crop species but also for different varieties of given crop (Singh and Agrawal 2007).

Material and Methods

Collection of soil samples

Twenty four bulk soil samples were collected from different locations of Anand district. The samples were air-dried, ground, and passed through a 2-mm sieve. These soils were analyzed for analysis of DTPA-extractable Ni content. On the basis of Ni content, 20 experimental soils were selected by broadly grouping them as 10 low (<0.5 mg Ni kg^{-1}), 6 medium (0.5–10 mg Ni kg^{-1}) and 4 high (>10 mg Ni kg^{-1}).

Pot experiment

A pot experiment was conducted in the net-house of Micronutrient Research Project, Anand Agricultural University, Anand, Gujarat. Six levels of Ni, *i.e.* Ni_0, Ni_2, Ni_4, Ni_6, Ni_8 and Ni_{10} (0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg Ni kg"1 soil) were applied in all 20 soils and triplicated, thus constituting a total of 360 (20 × 6 × 3) pots. Soil (5 kg) was filled in polythene lined earthen pots on air dry weight basis and incubated with respective treatments of Ni applied in solution through reagent grade nickel chloride ($NiCl_2.7H_2O$) (21% Ni). Six spinach seeds were sown in each pot and finally three seedlings were kept for further growth and recording dry matter yields.

Plant and soil analysis

The spinach crop was harvested three times in 15 days interval. In each cut the plant samples were sequentially washed with 0.1 *N* HCl, detergent solution and finally rinsed with deionized water, dried in hot air oven at 65 °C and the total plant dry matter yield was recorded. The dried plant samples were ground using stainless steel mechanical grinder. After grinding, 0.5 g plant material was digested using HNO_3 in microwave digester and final volume was made to 50 mL. Transparent digested material of plant and DTPA extractable Ni were determined by inductively coupled plasma (ICP-OES), Model Optima 7000 DV, Perkin Elmer, USA.

Determination of critical limit of Ni in soils and plant

The critical limit of Ni in soil was determined by Bray's per cent yield plotted against soil available Ni using the scattered diagram (Cate and Nelson 1965) and statistical procedure (Cate and Nelson 1971). Bray's per cent yield was calculated using following formula:

$$\text{Bray's per cent yield} = \frac{\text{Yield without Ni application}}{\text{Yield with optimum Ni level}} \times 100$$

The coefficient of determination (R^2) was calculated for determining critical level of deficiency of Ni by statistical procedure. The concentration having the highest R^2 is considered as the critical concentration of nutrient in question. The formula is given below:

$$R^2 = \frac{\text{TCSS} - (\text{CCS1} + \text{CSS2})}{\text{TCSS}} \times 100$$

where, TCSS = Total corrected sum of squares; CSS1 = Corrected sum of squares for population 1; CSS2 = Corrected sum of squares for population 2.

Statistical analysis

The data was analyzed statistically using analysis of variance with factorial completely randomized design for various parameters *viz.*, the amounts of Ni extracted by 0.005 *M* DTPA-$CaCl_2$, Bray's per cent yield, Ni concentration in spinach plant and its uptake were done using SPSS 16.0 (SPSS Institute Inc., Chicago, IL, USA).

Results and Discussion

pH, EC and Ni content of initial soils

The pH of the soils was neutral to alkaline in condition, whereas notable difference was found in case of electrical conductivity (EC). The pH of soils ranged from 6.66 to 9.14 and EC from 0.030 to 1.340 dS m^{-1}. Overall, pH and EC were 8.01 and 0.293 dS m^{-1}, respectively in all the experimental soils. There was a wide variation in the DTPA-extractable Ni content in soil and it ranged from 0.04 to 1.27 mg kg^{-1} with a average of 0.45 mg kg^{-1} across the soil (Table 1). This wide variations are because of variations in soil properties that affect available micronutrients distribution, including soil organic matter, pH, moisture regime, *etc*. (Zhu *et al.* 2016).

Dry matter produce (DMP) of spinach

Wide variation in spinach DMP was observed across the experimental soils (Table 2). The DMP of spinach in different soils varied from 9.60 to 12.39 g pot^{-1} for soil no. 1 to 10 (low Ni status soil), from 10.12 to 13.40 g pot^{-1} in soil no. 11 to 16 (medium Ni status soil), and from 10.24 to 12.81 g pot^{-1} in soil no.

Table 1. Initial pH, EC and DTPA-Ni content in soil

Soil No.	Ni status in soil	pH (1:2.5)	EC (dS m^{-1})	Ni (mg kg^{-1})
1*	Low	8.19	0.035	0.04
2		8.88	0.069	0.07
3		8.76	0.115	0.12
4		8.06	1.340	0.15
5		8.51	0.096	0.16
6		7.92	0.587	0.30
7		8.04	0.541	0.32
8		8.11	0.060	0.34
9		8.17	0.074	0.37
10		8.20	0.030	0.42
11	Medium	7.98	0.123	0.51
12		7.99	0.156	0.52
13		8.07	0.084	0.52
14		7.77	0.046	0.56
15		8.06	0.150	0.61
16		7.43	0.054	0.63
17	High	6.66	1.260	1.02
18		8.26	0.190	1.10
19		7.68	1.090	1.21
20		7.09	0.088	1.27
Mean		8.01	0.293	0.45
Range		6.66-9.14	0.030-1.340	0.04-1.27

*Low soil Ni group (soil no. 1–10): Medium soil Ni group (soil no. 11–16): High soil Ni group (soil no. 17–20),

17 to 20 (high Ni status soil). The effect of different levels of Ni application to spinach crop grown on these soils resulted in an increase of DMP up to 4.0 mg Ni kg^{-1} soil. The range and mean of DMP varied from 7.62 to 12.89 g pot^{-1} (mean 10.30 g pot^{-1}), 9.92 to 13.92 g (mean 11.67 g pot^{-1}), 9.50 to 14.42 g (mean 12.07 g pot^{-1}), 9.80 to 14.09 g (mean 11.98 g pot^{-1}), 9.19 to 12.98 (mean 11.40 g pot^{-1}) and 9.74 to 13.72 (mean 11.39 g pot^{-1}) with the application of 0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg Ni kg^{-1} soil, respectively (Table 2). The DMP of spinach did not increase at higher levels of Ni application (10.0 mg kg^{-1}). Results of the study revealed that the response by spinach to Ni application in increasing DMP is dependent on the initial Ni content in soil. The results obtained are in confirmation with the results reported by Ahmad *et al.* (2009) and Barman *et al.* (2014). They reported that high concentrations of Ni reduced yield in a number of crops including mung bean, oat, tomato and sunflower. The Bray's per cent yield ranged from 60.80 to 94.85 with a mean of 82.30. The absorption of Ni by plants was found dependent on the total content of Ni in soil (Roth *et al.* 1971) and physicochemical properties of the soil.

Table 2. The DMP of spinach as affected by different levels of Ni application in soil (g pot^{-1})

Soil No.	Ni status in soil	Nickel (mg kg^{-1}) 0	2.0	4.0	6.0	8.0	10.0	Mean	% Brays yield
1*	Low	8.31±1.0	9.92±1.4	10.19±1.3	9.99±1.9	9.19±0.9	9.98±1.9	9.60	81.5
2		10.90±0.6	12.79±0.3	14.06±1.5	11.13±0.8	11.79±0.5	10.96±0.6	11.94	77.4
3		7.62±1.1	12.45±0.2	12.38±1.1	11.75±0.4	12.53±0.9	10.21±1.1	11.16	60.8
4		8.93±1.0	11.83±1.4	9.50±1.2	12.33±1.3	9.70±1.0	9.86±0.9	10.36	72.3
5		9.54±1.2	11.67±1.6	9.80±0.7	11.01±1.8	11.81±1.1	10.25±1.2	10.68	80.7
6		10.67±1.7	10.55±1.5	11.24±0.2	12.19±1.1	12.58±1.3	12.14±0.8	11.56	84.8
7		9.89±1.4	11.16±0.6	13.06±0.5	11.43±1.9	9.91±1.3	10.76±2.2	11.03	75.7
8		10.55±1.1	12.27±0.8	13.42±1.4	13.06±1.3	12.67±1.8	12.37±0.7	12.39	78.6
9		10.08±1.4	10.01±0.4	11.17±0.9	12.13±1.0	12.03±0.6	11.38±0.2	11.13	83.0
10		9.60±1.5	11.20±1.3	11.25±1.3	9.99±1.6	9.59±1.3	12.20±0.8	10.64	78.6
11	Medium	11.09±1.7	10.84±1.0	12.57±1.1	12.17±0.4	12.48±1.6	11.15±1.8	11.72	88.2
12		12.25±1.5	13.26±1.3	14.21±0.6	12.65±1.0	12.44±0.5	13.40±0.1	13.04	86.1
13		11.33±1.0	11.65±0.6	12.24±1.0	11.69±1.6	11.79±1.3	11.46±1.1	11.69	92.5
14		10.18±0.4	11.31±0.2	10.76±0.9	10.80±1.4	10.42±1.0	11.23±1.1	10.78	90.0
15		11.74±0.4	13.92±0.9	13.30±0.7	13.53±0.8	12.98±1.9	13.00±0.8	13.08	84.3
16		9.78±0.3	10.01±0.0	11.01±0.5	10.10±0.4	9.84±2.0	10.12±0.4	10.15	88.8
17	High	12.89±1.1	12.68±0.5	13.59±1.7	12.46±0.7	11.92±1.6	11.62±1.2	12.53	94.8
18		9.72±0.4	10.75±0.5	10.88±1.6	9.80±0.2	10.54±1.2	9.74±0.3	10.24	89.3
19		9.89±0.3	12.74±0.9	14.42±0.7	14.09±1.8	12.02±0.8	13.72±1.8	12.81	68.5
20		11.07±0.5	12.36±1.9	12.42±1.0	11.19±1.0	11.68±1.6	12.18±0.3	11.82	89.1
Mean		10.30	11.67	12.07	11.68	11.40	11.39	11.42	82.3
Range		7.62-12.89	9.92-13.92	9.50-14.42	9.80-14.09	9.19-12.98	9.74-13.72		60.8-94.8

*Low soil Ni group (soil no. 1–10): Medium soil Ni group (soil no. 11–16): High soil Ni group (soil no. 17–20), ** standard error of mean of three replications

Ni content and uptake by plants

The mean Ni content in spinach plant ranged from 1.28 to 2.89 mg kg^{-1} in all the soils. Whereas, mean Ni uptake by spinach plants ranged from 10.65-37.25 µg pot^{-1} without application of Ni across the soils (Table 3). Similar results were also reported by Kumar *et al.* (2018b) who indicated that growth and yield attributes of barley increased up to the application of 10 mg Ni kg^{-1} soil whereas concentration and uptake of Ni increased with increasing levels of Ni supply.

Critical limit of Ni in soil and plant for spinach

The critical limit of deficiency of Ni for soils and spinach plant were worked out based on analysis of variance related to Bray's per cent yield and found almost similar values as determined by graphical and statistical methods as well (Fig. 1 and 2). In regard to the critical limit of Ni by graphical procedure as well as statistical procedure, the critical level (Table 4). In case of graphical method, critical limit of deficiency of DTPA-extractable Ni in soil and Ni content in plant were worked out as 0.50 and 2.20 mg kg^{-1}, respectively with graphical method. Narwal *et al.* (1991) reported positive response of corn to the Ni application in spite of corn containing 2.5 mg kg^{-1} of Ni on dry weight basis in control. Several workers

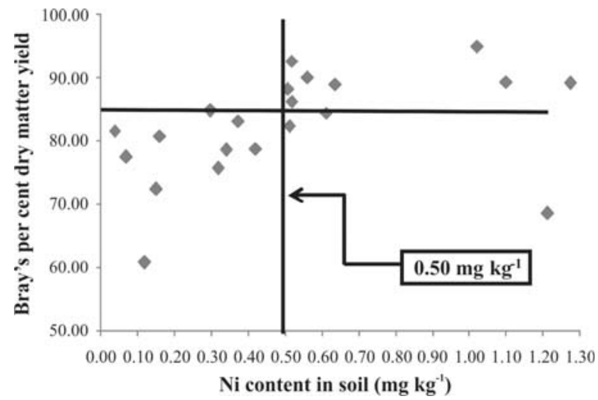

Fig. 1. DTPA-extractable Ni (mg kg^{-1}) in soil

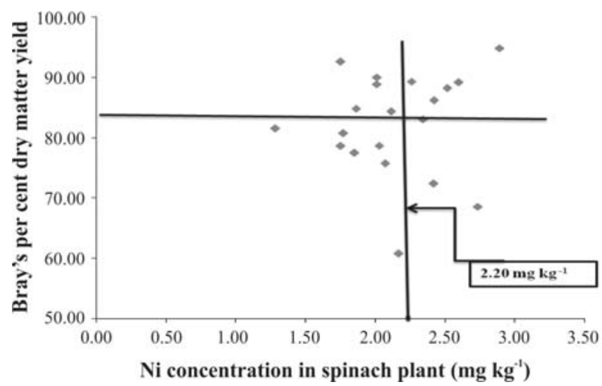

Fig. 2. Nickel concentration in spinach plant (mg kg^{-1})

Table 3. The DMP of spinach, Ni content and uptake by the crop under control (Without Ni Application)

Soil No.	Ni Status in soil	DMP (g pot^{-1})	Ni concentration (mg kg^{-1}) in plant	Ni uptake (µg pot^{-1}) by plant
1	Low	8.31±1.0	1.28±0.2	10.6±2.9
2		10.90±0.6	1.85±0.1	20.2±0.5
3		7.62±1.1	2.17±0.1	16.5±3.2
4		8.93±1.0	2.42±0.1	21.6±3.3
5		9.54±1.2	1.77±0.2	16.9±3.9
6		10.67±1.7	1.86±0.2	19.9±2.0
7		9.89±1.4	2.07±0.4	20.5±4.8
8		10.55±1.1	1.75±0.3	18.5±5.2
9		10.08±1.4	2.34±0.2	23.6±4.7
10		9.60±1.5	2.03±0.1	19.5±3.1
11	Medium	11.09±1.7	2.52±0.2	27.9±4.4
12		12.25±1.5	2.42±0.1	29.6±4.8
13		11.33±1.0	1.75±0.2	19.8±3.9
14		10.18±0.4	2.01±0.0	20.5±1.1
15		11.74±0.4	2.11±0.3	24.8±2.3
16		9.78±0.3	2.01±0.3	19.7±3.3
17	High	12.89±1.1	2.89±0.3	37.3±6.2
18		9.72±0.4	2.26±0.1	22.0±1.7
19		9.89±0.3	2.74±0.1	27.1±0.7
20		11.07±0.5	2.60±0.1	28.7±2.2
Mean		10.30	2.14	22.25
Range		7.62-12.89	1.28-2.89	10.65-37.25

*Low soil Ni group (soil no. 1–10): Medium soil Ni group (soil no. 11–16): High soil Ni group (soil no. 17–20), ** standard error of mean of three replications

Table 4. Statistical method for computation of critical limit of deficiency of nickel content (mg kg^{-1}) in soil and spinach plant

Soil No.	Ni content in soil (mg kg^{-1})	Ni content in spinach (mg kg^{-1})	% Bray's yield of spinach	r^2 for soil	r^2 for plant
1	0.04	1.28	81.5	0.167	0.169
2	0.07	1.85	77.4	0.189	0.179
3	0.12	2.17	60.8	0.403	0.343
4	0.15	2.42	72.3	0.517	0.428
5	0.16	1.77	80.7	0.511	0.409
6	0.30	1.86	84.8	0.462	0.356
7	0.32	2.07	75.7	0.546	0.411
8	0.34	1.75	78.6	0.602	0.440
9	0.37	2.34	83.0	0.601	0.423
10	0.42	2.03	78.6	0.675*	0.462*
11	0.51	2.52	88.2	0.615	0.398
12	0.52	2.42	86.1	0.596	0.364
13	0.52	1.75	92.5	0.495	0.281
14	0.56	2.01	90.0	0.440	0.234
15	0.61	2.11	84.3	0.467	0.228
16	0.63	2.01	88.8	0.444	0.199
17	1.02	12.89	94.8	0.345	0.168
18	1.10	2.26	89.3	0.252	0.184
19	1.21	2.74	68.5		
20	1.27	2.60	89.1		

Critical limit for plant = (2.03 + 2.52)/2 = 2.27
Critical limit for soil = (0.42+0.51)/2 = 0.46
*Critical limit is computed as the mean value of Ni content in soil having maximum value of R^2 and that in succeeding soil and plant as well.

have also done the work to find out the critical limit of Ni in different crops (Wood and Nyczepir 2004; Kumar *et al.* 2018a).

Conclusions

Positive response on spinach drymatter production was noticed when Ni was applied at the rate of 4.0 mg Ni kg^{-1} soil. Different levels of Ni application caused a significant increase in Ni concentration in shoot as well as uptake in spinach plant in all the soils. According to the findings, 0.005 *M* DTPA-$CaCl_2$ extractable critical limit of Ni in soil and plant was worked out as 0.46 and 2.27 mg kg^{-1}, respectively, by statistical procedure. Similarly, in graphical procedure, it was 0.50 and 2.20 mg kg^{-1}, respectively, using the spinach as test crop.

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