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Low Concentration of Cobalt Increases Growth, Biochemical Constituents, Mineral Status and Yield in *Zea Mays*

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Abstract

A pot culture experiment has been carried out to find the extent of changes occurring in the biochemicals and nutrients of maize plants (*Zea mays* L.) grown under different concentrations of cobalt (50, 100, 150, 200, 250 mg kg^{-1} soil). The growth and yield parameters such as seedling vigour, number of cobs, number of seeds per plant; photosynthetic pigments viz., chlorophyll 'a', chlorophyll 'b', and total chlorophyll contents; biochemicals like total sugars (reducing and non reducing), starch, amino acids and protein content and various macro- and micronutrients are determined 90 days after sowing (DAS). All the growth parameters, pigment content, biochemicals and mineral content increase at 50 mg Co kg^{-1} soil when compared with the control. Further increase in the Co levels (100-200 mg kg^{-1} soil) has a negative effect on all the above parameters.

Keywords: Cobalt; Growth; *Zea mays*; Biochemicals; Nutrients.

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1. Introduction

The concentration of heavy metals in air, water and soil leads to many hazardous effects to living organisms. With the development of industries, mining activities, application of wastewater and sewage sludge on land, heavy metal pollution of soils is increasingly becoming a serious environmental problem. Excessive metal concentrations in contaminated soils can result in decreased soil microbial activity and soil fertility and crop yield losses [1]. Accumulation of trace elements, especially heavy metals, in the soil has potential to restrict the soil's function, cause toxicity to plants, and contaminate the food chain [2].

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Cobalt (Co) is known to cause irreversible damage to a number of vital metabolic constituents and plant cell and cell membrane. While it has been known for many years that Co is an essential element for humans, animals and prokaryotes, a physiological function for this element in higher plants has not been identified. The Co-containing vitamin B_{12} does not occur in plants. Whereas normal Co concentrations in plants are cited to be as low as 0.1-10 μg g^{-1} dry weights, its beneficial role as a trace element has been described [3]. Trace elements are necessary for the normal metabolic functions of the plant, but at higher concentrations, these metals are toxic and may severely interfere with physiological and biochemical functions [4-6].

Plants under stress produce some defence mechanisms to protect themselves from the harmful effect of oxidative stress. The plants metal response will result in the production of reactive oxygen species (ROS), which leads to the activation of defense mechanisms, in terms of antioxidant enzymes [7]. Thus, ROS scavenging is one among the common defense responses against abiotic stresses [8]. The present investigation was executed with an objective to study the effects of Co stress on growth, nutrients content and biochemical constituents of *Zea mays* L.

2. Materials and Methods

2.1. *Plant materials and cultivation*

The seeds of maize (*Zea mays* L.) were obtained from Tamil Nadu Agricultural University, Tamil Nadu, India and surface sterilized with 0.1% $HgCl_2$ solution for 1 min with frequent shaking and then thoroughly washed with demonized water. Plants were grown in pots in untreated soil (control) and in soil to which Co had been applied (50, 100, 150, 200 and 250 mg kg^{-1} soil). The inner surface of pots was lined with polythene sheet. Each pot contained 13 kg of air-dried soil. The Co as finely powdered ($CoCl_2$) was applied to the surface soil and thoroughly mixed with the soil. Five seeds were sown in each pot. All the pots were watered to field capacity daily. After a week of germination, plants were thinned to a maximum three per pot. Each treatment including control was replicated six times. The plant samples were collected on 90 days after sowing (DAS) for the measurement of various growth parameters, biochemical, nutrients contents and antioxidant enzyme activities.

2.2. *Morphological and yield parameters*

The growth parameters like root and shoot lengths, number of cobs per plant and number of seeds per plant were measured in the samples.

2.3. *Biochemical analyses*

The biochemical analysis such as chlorophyll content [9], amino acids [10] and protein content [11] were carried out in fresh samples. Starch was extracted and

estimated following the method of Lustinec *et al.* [12]. Soluble sugars were estimated using the method of Copp *et al.* [13] and expressed in mg g^{-1} dry weight.

2.4. *Nutrient content estimations*

2.4.1. *Estimation of total nitrogen [14]*

Briefly, dried plant materials (100 mg) were digested in 5 ml of salicylic-sulphuric acid mixture (5g salicylic acid in 100 ml concentrated sulphuric acid). After 30 min, approximately 0.3g sodium thiosulphate was added and heated gently until fumes appeared. Then 5 ml of concentrated sulphuric acid and approximately 0.1 g of catalyst mixture (Copper sulphate, potassium sulphate and selenium dioxide mixed in the ratio of 1:8:1) were added. Digestion was continued for at least 3 h, till the digest has become colourless. On completion of digestion, the flask was cooled and content diluted to 50 ml in volumetric flask using distilled water.

Distillation of digested content was continued until 30 ml distillate had been collected. Then the whole distillate was titrated against standard 1/28 hydrochloric acid solution until the pink colour just reappeared. Blank digestion, distillation and titration were made using all the reagents without plant sample.

The percentage of total nitrogen was calculated by the following formula:

$$\text{Percentage of nitrogen} = (T - B) \times 5 \times N \times 1.4/S$$

where, T = Sample titrated (ml); B = Blank titrated (ml);
N = Normality of hydrochloric acid (1/28 = 0.036);
S = Weight of plant material (g); Aliquot factor = 5

2.4.2. *Estimation of phosphorus [15]*

One gram of dried and ground plant tissue was digested with 10 ml of acid mixture (nitric acid, 750 ml; sulphuric acid, 150 ml; perchloric acid 60%, 300 ml). The digest was cooled and made unto 50 ml and filtered through acid washed Whatman No.1 filter paper. One ml of digest was mixed with 2 ml of 2 N nitric acid and diluted to 8 ml. One ml of molybdovanadate reagent (25 g of ammonium molybdate in 500 ml water, 1.25g ammonium vanadate in 500 ml of 1 N nitric acid; both were mixed in equal volumes) was added, make up to 10 ml, shacked and the absorbance was measured at 420 nm in a spectrophotometer, after 20 minutes of standing. Standard graph was prepared using potassium dihydrogen phosphate.

2.4.3. *Estimation of potassium [16]*

Dried and ground tissues weighing 0.5 g were digested in 100 ml Kjeldahl flasks using 15 ml of concentrated nitric acid, 0.5 ml of 60% perchloric acid and 0.5 ml of concentrated sulphuric acid. Digestion was continued until the nitric and perchloric acids were driven-

off. The inorganic residue was cooled and diluted with 15 ml of distilled water and filtered through Whatman No. 42 filter paper. The filtrate was made up to 50 ml with distilled water. The filtrate was used for potassium estimation by flame photometer and standards were prepared with potassium chloride.

2.4.4. *Estimation of copper, iron, manganese and zinc [17]*

One ml of sulphuric acid and 15 ml of double distilled water were added to a Kjeldahl flask containing 0.5 g of dried and powdered material and incubated at 80°C for over a night. After that 5 ml of acid mixture (nitric acid, 3: perchloric acid, 1) was added and digested until the nitric acid and perchloric acid were driven off. The digest was cooled, diluted, filtered through Whatman No. 42 filter paper and made up to 50 ml. The solution was directly aspirated to an Atomic Absorption Spectrophotometer (Perkin - Elmer - 2280), with air/acetylene flame for estimating copper, iron, manganese and zinc.

2.5. *Statistical analysis*

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values are mean ± SD for six samples in each group. *P* values ≤ 0.05 were considered as significant.

3. Results and Discussion

3.1. *Growth parameters*

The seedling vigour of *Z. mays* plants at 90 DAS under Co treatment is represented in Table 1. The seedling vigour of *Z. mays* decreased with the increase in Co concentrations in the soil. The highest root and shoot length of *Z. mays* was observed at 50 mg kg^{-1} on 90 DAS and lowest root and shoot length was observed at 250 mg kg^{-1} on 90 DAS.

Similar decrease in plant height was reported previously [6]. Cobalt at high levels may inhibit the root and shoot growth directly by inhibition of cell division or cell elongation or combination of both, resulting in the limited exploration of the soil volume for uptake and translocation of nutrients and water and induced mineral deficiency [18].

3.2. *Yield parameters*

The yield parameters like number of cobs and number of seeds per plant varied significantly due to the metal treatment in *Z. mays*. The maximum number of cobs per plant and number of seeds per plant occurred in 50 mg kg^{-1} Co treatment on 90 DAS (Table 1). The minimum number of cobs per plant and number of seeds per plant were observed at 250 mg kg^{-1} Co. The number of cobs and number of seeds per plant of *Z.*

mays decreased with the increase in cobalt level in the soil. For low level of cobalt, the yield parameters recorded were higher at 50 mg kg^{-1} Co when compared with the control, and thus, this concentration showed a beneficial effect on maize plants that is in consonance with the findings of Barik and Chandel [19] and Saravanan *et al.* [20] in soybean and Sharma and Sharma [21] in case of wheat.

Table 1. Effects of cobalt on some morphological and yield parameters of *Z. mays.*

Co added in soil (mg kg^{-1})	Root length (cm)	Shoot length (cm)	Number of cobs $plant^{-1}$	Seeds $plant^{-1}$
Control	97±0.554	153±.494	5.06±0.15	461±13.83
50	113±0.640	161±0.561	6.15±0.18	588±17.64
100	85±0.410	147±0.434	4.22±0.13	444±13.32
150	76±0.314	131±0.402	3.48±0.10	420±12.60
200	69±0.215	112±0.320	2.79±0.84	360±10.80
250	61±0.192	88±0.263	1.79±0.54	311± 9.33

Values are mean±SD within each group (n=6).

3.3. *Biochemical constituents*

Photosynthetic pigments such as chlorophyll ‘a’, chlorophyll ‘b’, and total chlorophyll content of *Z. mays* leaves increased at lower concentration (50 mg kg^{-1}) (Fig. 1). Sugars (total sugars, reducing, reducing, nonreducing sugars), starch, amino acids, and protein contents of Z. mays increased at 50 mg Co kg^{-1} soil and decreased with further increase in the Co levels (100-250 mg kg^{-1}; Fig. 2 and 3).

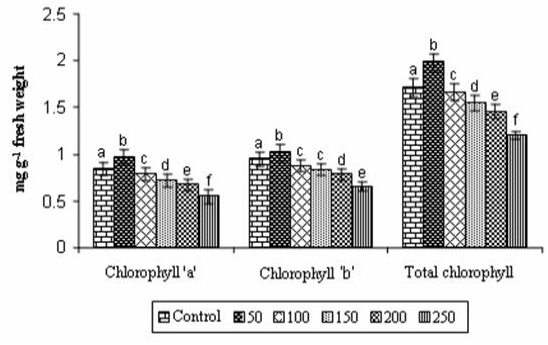

Fig. 1. Cobalt induced changes in photosynthetic pigment contents of *Z. mays*. Values are mean ± SD represented by vertical bars (n = 6). Values not sharing the same superscripts are significantly different ($P \leq 0.05$).

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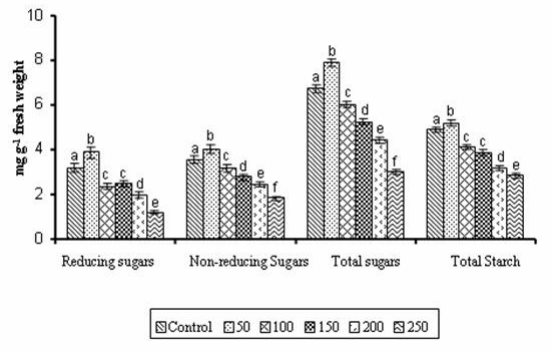

Fig. 2. Cobalt induced changes in sugars (total sugars, reducing and non reducing sugars) and starch contents of *Z. mays*. Values are mean ± SD represented by vertical bars (n = 6). Values not sharing the same superscripts are significantly different ($P \leq 0.05$).

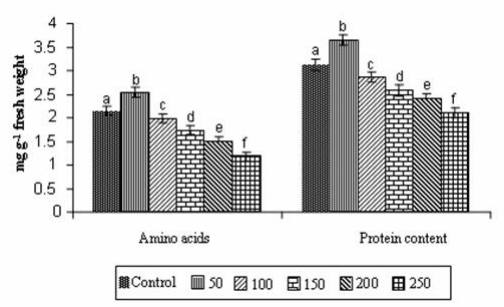

Fig. 3. Cobalt induced changes in amino acid and protein contents of *Z. mays*. Values are mean ± SD represented by vertical bars (n = 6). Values not sharing the same superscripts are significantly different ($P \leq 0.05$).

Photosynthetic pigments such as chlorophyll ‘a’, chlorophyll ‘b’, and total chlorophyll contents of *Z. mays* decreased with increasing Co level in the soil. Similar changes in the content by various metal treatments were recorded [22]. The increased chlorophyll content at lower level of Co was obviously due to better growth.

The increase in Co levels showed a marked depression in photosynthetic pigments in maize plants. It might be due to excess supply of Co resulting in interference with the synthesis of chlorophyll. The formation of chlorophyll pigment depends on the adequate supply of metal ions [23]. The excess supply of Co seems to prevent the incorporation of iron in protoporphyrin molecule resulting in the reduction of chlorophyll pigment. This was strengthened by the fact that excessive amounts of a range of heavy metals such as cobalt [4], nickel [24] induced chlorosis in plants, which were usually similar to the chlorosis of iron deficiency. Impaired chlorophyll development by heavy metals may be due to the interference to protein; the treatments presumably blocked the synthesis and activities of enzyme proteins responsible for chlorophyll biosynthesis.

Sugars (total sugars, reducing and non reducing sugars) and starch content of plants showed a decreasing trend with progressive increase in Co level in the soil, however, 50 mg kg^{-1} Co level produced positive effect on the sugar and starch contents which is in consonance with the findings of Swamy and Theresa [25] in *Phaseolus mungo*. The accumulation of total sugar and starch decreased with increase in Co level. Considerable physiological changes have been observed in crops grown in soil contaminated with even moderate level of metals [26]. In order to obtain a better understanding of the physiological effect of the Co on chlorophylls, sugars, starch, amino acids and protein content we also determined these parameters.

Co level over 50 mg kg^{-1} soil significantly reduced the amino acid and protein content in the leaves of *Z. mays*. Nitrogen is a precursor for the synthesis of amino acids, since the nitrogen content of the metal treated plants was found reduced, ultimately amino acids and protein contents of plants were also reduced, suggesting that there was only a limited availability of nitrogen for the synthesis of amino acids.

Co at 50 mg kg^{-1} soil level increased the amino acid and protein contents of *Z. mays*. Kleizaite *et al.* [2] observed similar trends due to application of heavy metals like copper, zinc, mercury, lead and cadmium in rice. Further increase in Co level decreased the amino acid and protein contents. These results were strengthened by the findings of Parmer and Chanda [5] who also reported cadmium and lead mediated decrease in these parameters in *Vigna unguiculata* (L.) Walp.

3.4. *Nutrients*

3.4.1. *Macronutrients (mg g^{-1} dry wt.)*

Nitrogen, phosphorus and potassium content of leaves of *Z. mays* is represented in Table 2. Nitrogen, phosphorus and potassium content of leaves was higher at 50 mg kg^{-1} Co level and decreased with further increase in Co level in the soil. The lowest nitrogen, phosphorus and potassium content of *Z. mays* leaves was recorded at 250 mg Co kg^{-1} soil.

Different Co levels altered nitrogen content in *Z. mays*. However, 50 mg kg^{-1} Co level produced positive effect on the nitrogen content of *Z. mays*. The reduction in nitrogen content under Co treatment was comparable with the results of Swamy and Theresa [25] in cadmium, lead and zinc treatment. Mocquot *et al.* [27] emphasized that heavy metals sharply decrease the NO_3 uptake by roots, incorporation of nitrogen into organic compounds and translocations of nitrogen to leaves. Phosphorus content of *Z. mays* plants decreased with an increase in the Co levels (except 50 mg kg^{-1}) in the soil. Excess amount of trace elements could affect the mineral nutrition of plants. Metal toxicity may affect certain elements more than others and interactions among elements may occur [24]. Here the phosphorus content of leaves of *Z. mays* plants decreased due to Co stress.

Cobalt level above 50 mg kg^{-1} significantly reduced the potassium content in leaves of *Z. mays*. Heavy metal toxicity in general has been associated with the reduced absorption and accumulation of potassium [28]. Potassium is one of the essential macro nutrients, taken up by the roots and generally transported to shoot through the xylem, and this transport seems to be controlled by the shoot growth [29]. Therefore, decreased potassium

content of *Z. mays* due to Co may be due to the toxic effect of Co on plant growth or competition by other ions, which in turn exercised a regulatory control on potassium uptake.

3.4.2. *Micronutrients (μg g^{-1} dry wt.)*

Copper, iron, manganese and zinc content of leaves of *Z. mays* is presented in Table 2. Maximum copper, iron, manganese and zinc contents in leaves of *Z. mays* were recorded at 50 mg Co kg^{-1} soil. The minimum Copper, iron, manganese and zinc contents of *Z. mays* leaves were observed at 250 mg Co kg^{-1} soil.

Table 2. Effects of cobalt stress on macro- and micronutrients of *Z. mays*.

Cobalt	N	P	K	Cu	Fe	Mn	Zn
(mg kg^{-1})	mg g^{-1} dry weight			μg g^{-1} dry weight			
0	25.2 ±0.771	28.4 ± 0.872	21.3 ± 0.659	17 ± 0.551	226 ± 6.140	154 ± 4.720	36.8 ± 1.012
50	27.5± 0.875	30.7 ± 0.941	23.1 ± 0.713	20 ± 0.620	275 ± 7.550	176 ± 5.380	38.7± 1.091
100	22.4± 0.701	27 ± 0.830	20.2 ± 0.626	16 ± 0.512	211 ± 5.390	133 ± 4.190	31.0± 0.860
150	18.8± 0.584	24.3 ± 0.749	17.5 ± 0.545	14 ± 0.446	174 ± 4.520	115 ± 3.550	26.1± 0.713
200	16.5± 0.515	21.1 ± 0.653	15.3 ± 0.479	12 ± 0.389	139 ± 4.370	102 ± 3.060	22.5± 0.605
250	14.0± 0.440	15.7 ± 0.502	13.0 ± 0.410	09± 0.285	83 ± 2.590	75 ± 2.350	19 ± 0.580

Values are means ± SD within each group (n = 6).

Increased Co content in the soil significantly decreased the copper content of leaves of *Z. mays*. However, low level of Co (50 mg kg^{-1} soil) increased the copper content. The decrease in copper content of the plant tissues studied can be explained to some extent by the close association of copper with nitrogen ligands [4]. It has been reported that there was a close and parallel relation in the movement of copper and nitrogen in the plants [30]. In the present investigation, the decrease in the copper content was parallel with the decrease in nitrogen and potassium concentrations.

Iron content of *Z. mays* decreased with increase in Co content in the soil. These results are in agreement with the results using nickel treatment [31]. El-Sheekh *et al.* [4] found that the inhibition of iron absorption was due to occupation of interfering ions in iron absorbing sites. Therefore, the efficient translocation of iron appears to depend on the chelation of such interfering ions by organic acids in the plant.

Manganese and zinc content of leaves of *Z. mays* plants under Co treatment decreased gradually with increase in Co level in the soil. However, 50 mg Co kg^{-1} soil

exhibited higher manganese and zinc content in maize leaves. Inhibition of manganese and zinc uptake was in accordance with the findings of Lidon and Henriques [29] using copper and Moral *et al.* [32] in cadmium treatment.

In conclusion, Co treatment at 50 mg kg^{-1} soil increased various growth and yield parameters, pigment content, total sugars, starch, amino acids and protein content including various other nutrients in the leaves of *Z. mays*. Therefore, it can be suggested that 50 mg Co kg^{-1} soil is beneficial for the growth of *Z. mays* plants.

References

1. S. N. Whiting, M. R. Broadley, and P. J. White, Plant Soil **249**, 45 (2003). doi:10.1023/A:1022542725880
2. V. Kleizaite, T. Cesniene, and V. Rancelis, Plant Sci. **167**, 1249 (2004). doi:10.1016/j.plantsci.2004.06.023
3. E. Bakkaus, B. Gouget, J. P. Gallien, H. Khodja, F. Carrot, J. L. Morel, and R. Collins, Nucl. Instr. Meth. Phys. Res. B. **231**, 350 (2005). doi:10.1016/j.nimb.2005.01.082
4. M. M. El-Sheekh, A. H. El-Naggar, M. E. H. Osman, and E. El-Mazaly, Braz. J. Plant Physiol. **15**, 159 (2003). doi:10.1590/S1677-04202003000300005
5. G. Parmar and V. Chanda, Turk. J. Biol. **29**, 15 (2005).
6. K. Jayakumar, P. Vijayarengan, Zhao Chang-Xing, and C.A. Jaleel, Colloids Surf. B: Biointerf. **67**, 272 (2008). doi:10.1016/j.colsurfb.2008.08.012
7. J. A. Imlay, Annu. Rev. Microbiol. **57**, 395 (2003). doi:10.1146/annurev.micro.57.030502.090938
8. E. Vranova, D. Inze, and F. Van Brensegem, J. Exp. Bot. **53**, 1227 (2002). doi:10.1093/jexbot/53.372.1227
9. D. I. Arnon, Plant Physiol. **24**, 1 (1949). doi:10.1104/pp.24.1.1
10. S. Moore and W. H. Stein, J. Biol. Chem. **176**, 367 (1948).
11. M. M. Bradford, Anal. Biochem. **72**, 248 (1976). doi:10.1016/0003-2697(76)90527-3
12. J. Lustinec, V. Hadadova, and M. Kaminck, Z. Prochazka, Anal. Biochem. **132**, 265 (1983). doi:10.1016/0003-2697(83)90006-4
13. L. J. R.W. Copp, R. Y. Blenkinsop, and A. G. Yada, Am. J. Potato Res. **77**, 279 (2000).
14. K. Peach and M. V. Tracey, Modern Methods of Plant Analysis, Vol. I. Springer Verlag, Berlin (1956) p. 477.
15. S. Yoshida, D. A. Forno, J. Cock, and K. A. Gomez, Laboratory Manual for Physiological Studies of Rice, IRRI, Philippines (1972).
16. C. H. Williams and V. Twine, In: *Modern Methods of Plant Analysis* (eds.). K. Peach and M. V. Tracey. Vol. **V**. Springer Verlag, Berlin (1960) pp. 3-5.
17. M. P. C. DeVries and K.G. Tiller, Commonwealth Scient. Ind. Res. Org. Aus (1980).
18. A. Hemantaranjan, A. K. Trivedi, and M. Ram, Indian J. Plant Physiol. **5**, 142 (2000).
19. K. C. Barik and A. S. Chandel, Ind. J. Agri., **46**, 319 (2001).
20. S. Saravanan, A. Subramani, P. Sundramoorthy, M. Selvaraj, and A. S. Lakshmanachary, Ecol. Environ. Cons. **7**, 141 (2001).
21. D. C. Sharma and C. P. Sharma, Cereal Res. Commun. **21**, 317 (1993).
22. G. R. Rout and P. Das, Agronomie, **23**, 3 (2003). doi:10.1051/agro:2002073
23. M. Nyitrai, A. G. Szent-Györgyi, and M.A. Geeves, Biochem. J. **365**, 19 (2002). doi:10.1042/BJ20020099
24. P. Vijayarengan and D. Dhanavel, Adv. Plant Sci. **18**, 253 (2005).
25. M. Swamy and Y. M. Theresa, Geobios. **19**, 242 (1992).
26. A. Mithöfer, B. Schulze, and W. Boland, FEBS Letters **566**, 1 (2004). doi:10.1016/j.febslet.2004.04.011
27. B. Mocquot, J. Vangronsveld, J. Clijestres, and H. Menchn, Plant Soil **182**, 287 (1996).

28. Y. M. Siddiqui and A. D. M. Glass, Can. J. Bot. **61**, 1551 (1983).
29. F. C. Lidon and F. S. Henriques, Bot. Bull. Acad. Sinica. **33**, 141 (1992).
30. S. P. McGrath, A. M. Chaudri, K. E. Giller, J. Indust. Microbiol. **14**, 94 (1995). doi:10.1007/BF01569890
31. P. Vijayarengan, A. S. Lakshmanachary, Indian J. Environ. Health **37**, 99 (1995).
32. R. Moral, J. Gomez, N. Pedreno, and J. Mataix, J. Plant Nutr. **17**, 953 (1994). doi:10.1080/01904169409364780
33. S. N. Whiting, M. R. Broadley, and P. J. White, Plant Soil **249**, 45 (2003).