Amer. J. Bot. 70(8): 1254–1257. 1983.

USE OF THE DCB TECHNIQUE FOR EXTRACTION OF HYDROUS IRON OXIDES FROM ROOTS OF WETLAND PLANTS[1]

GREGORY J. TAYLOR AND A. A. CROWDER

Department of Biology, Queen's University, Kingston, Ontario K7L 3N6, Canada

ABSTRACT

Replicate samples of *Phragmites communis* roots coated with hydrous iron oxide were analyzed for internal and external Fe using the dithionite-citrate-bicarbonate technique (hot DCB), a modified DCB technique (cold DCB), Ethylenediamine tetraacetic acid (EDTA), Diethylenetriamine pentaacetic acid (DTPA), and a total Fe analysis. EDTA and DTPA were found to be inefficient in extracting FeOOH while both DCB techniques were efficient. Concentrations of Fe extracted (8.93% and 9.37% by weight) and percent of total Fe extracted (98.3% and 98.6%) by the hot and cold DCB techniques (respectively) were not significantly different. Results of the total Fe analysis (8.58% Fe by weight) were the same as extracted Fe and total Fe determined by the DCB techniques. This may reflect the large external Fe : internal Fe ratio of the sample roots. Despite equivalent results, the cold DCB technique is preferred over the hot DCB technique due to a reduced likelihood of structural damage to roots.

A NUMBER OF WETLAND PLANTS have been shown to oxidize the rhizosphere, a process which may serve to protect against the entry of reduced phytotoxins such as Mn^{2+}, Fe^{2+}, and S^{2-} (Armstrong, 1967, 1972, 1978). A result of oxidation of the rhizosphere is the formation of Fe coatings, or plaques, shown to be α-FeOOH (goethite) and α-FeOOH (lepidocrocite) by Chen, Dixon and Turner (1980) and Bacha and Hossner (1977). Recent studies of these plaques have led to the application of the dithionite-citrate-bicarbonate (DCB) technique. While this technique was designed for the extraction of iron oxides from soil material (Jackson, 1956), it is now used for the extraction of iron oxides from plant roots (for example, see Bacha and Hossner, 1977; Chen, Dixon and Turner, 1980; Mendelssohn and Postek, 1982). Few other techniques have been utilized (see for example, Foy, Fleming and Schwartz, 1981).

In preliminary experiments with *Typha latifolia* L. grown under laboratory conditions, roots extracted with the DCB technique showed considerable structural damage and retained little Fe in washed tissues. To overcome this problem, the extraction procedure was slightly modified and this resulted in less apparent physical damage. The DCB technique has not been critically evaluated and techniques for the preparation of roots for DCB extraction have not been standardized. Differences in root preparation may affect the amount of structural damage and hence the proportion of root Fe extracted. This study compares the effectiveness of the DCB technique, the modified DCB technique, and the synthetic chelates ethylenediamine tetraacetic acid (EDTA) and diethylenetriamine pentaacetic acid (DTPA) for extracting FeOOH from plant roots.

MATERIALS AND METHODS—Roots of *Phragmites communis* Trin. were collected from a wetland site on a railway right-of-way in Bedford township, Frontenac County, Ontario. The uppermost roots were completely covered with a reddish-brown deposit, while the lower roots were black, presumably due to a coating of a sulfide material. Root material covered with the reddish-brown deposits was collected and immediately returned to the lab. The roots were hand-washed in tap water, immersed in cold tap water (9.5 C) overnight, and rinsed twice with deionized water. The roots were then divided into 95 portions of roughly equal weight (approx. 0.2 g dry wt) and randomly assigned to one of five extraction techniques described below. All extraction techniques requiring fresh roots were completed in less than 24 hr from the time the roots were collected.

a) Total extraction: Roots were dried to constant weight at 60 C and digested for 12 hr at 120 C with 2 ml HNO_3 and 1 ml H_2O_2 in Teflon pressure chambers. The chambers were then washed twice with deionized water and the resulting solution was made to volume (25 ml) with deionized water.

b) Hot DCB extraction: Fresh roots were ex-

[1] Received for publication 10 January 1983; revision accepted 1 June 1983.

This study was supported by funds from the National Research Council of Canada (Contract number OSU82-00224) and the Queen's University Advisory Research Committee. We would like to thank Sheila Macfie for assistance in the field.

TABLE 1. *Mean ± standard error of total sample weight (dry weight + extracted FeOOH), extracted Fe (external Fe), root Fe (internal Fe), total Fe and percent of total Fe extracted*

Variable	Extraction Procedures[a]				
	Total	Hot DCB	Cold DCB	EDTA	DTPA
Weight (g)	0.183 ± 0.007a	0.169 ± .008ab	0.176 ± .006ab	0.158 ± 0.006b	0.173 ± 0.004ab
Extracted Fe (% by wt)	—	8.93 ± 0.50a	9.37 ± 0.59a	5.33 ± 0.21b	0.71 ± 0.06c
Root Fe (% by wt)	8.58 ± 0.39a	0.14 ± 0.01b	0.12 ± 0.01b	4.72 ± 0.34c	7.32 ± 0.31d
Total Fe (% by wt)	8.58 ± 0.39ab	9.08 ± 0.50abc	9.49 ± 0.59ac	10.05 ± 0.43c	8.03 ± 0.36b
% of total Fe extr.	—	98.3 ± 0.12a	98.6 ± 0.14a	53.7 ± 1.76b	8.7 ± 0.58c

[a] Letters to the right of each mean value indicate groups of means that do not differ at the 5% level (Duncan's Multiple Range test).

tracted with the technique reported by Bacha and Hossner (1977), Chen, Dixon and Turner (1980) and Mendelssohn and Postek (1982). However, Chen, Dixon and Turner (1980) used $^1/_5$ strength DCB solution. Roots were placed in a solution of 40 ml of 0.3 M sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) and 5 ml of 1.0 M sodium bicarbonate ($NaHCO_3$) at 75 C. One gram of sodium dithionite ($Na_2S_2O_4$) was added and the mixture was agitated constantly for 5 min. Two additional 1-g portions of $Na_2S_2O_4$ were added, each followed by a 5-min agitation period. After the 15-min extraction period, the wash was collected and the roots were washed 3 times with 15 ml of deionized water. The resulting solution was made to volume (100 ml) with deionized water. To determine the amount of Fe remaining in the roots after washing, the root material was then dried to constant weight at 60 C and the washed root material was prepared for Fe determination as in (*a*) above.

c) *Cold DCB extraction:* Fresh roots were extracted with cold DCB, a modification of the technique in (*b*) above. Roots were agitated for 3 hr in a solution of 40 ml of 0.3 M $Na_3C_6H_5O_7 \cdot 2H_2O$, 5 ml of 1.0 M $NaHCO_3$ and 3 g of $Na_2S_2O_4$ at room temperature (21 C). The wash was collected and the roots washed 3 times with 15 ml of deionized water. The resulting solution was made to volume (100 ml) with deionized water and the washed roots were prepared for Fe determination as in (*a*) above.

d) *EDTA extraction:* Fresh roots were extracted in a manner similar to that described by Foy, Fleming and Schwartz (1981). Fresh root material was agitated in 45 ml of 0.005 M EDTA for 3 hr at room temperature. The wash was collected and made to volume (100 ml) with deionized water. The washed roots were prepared as in (*a*) above.

e) *DTPA extraction:* Fresh roots were extracted with 45 ml of 0.005 M DTPA, 0.01 M $CaCl_2$, and 0.1 M Triethanolamine (pH 7.3) (Lindsay and Norvell, 1978) at room temperature for 3 hr. The wash was collected and made to volume (100 ml) with deionized water. The washed roots were prepared as in (*a*) above.

For all samples, Fe was determined by atomic absorption spectrophotometry. DCB solutions produced considerable interference with the absorption of the Fe wavelenth, so the spectrophotometer was calibrated with Fe standards prepared with 45 ml of the DCB solution made to volume (100 ml) with deionized water. These standards were unstable over long periods of time, hence the $Na_2S_2O_4$ was substituted with sodium sulfite (Na_2SO_3) in the same molar concentration with respect to Na. Standards prepared in this manner mimicked the interference of the DCB solutions and were stable for long term storage. Sample dilution decreased, but did not eliminate, interference. Hence, when sample dilution was required for the DCB analysis, standards with equal dilution ratios were also prepared. Preparation of these standards has not been reported elsewhere in the literature, and failure to prepare suitable standards may result in errors approaching 100% as referenced to the total extractions. Concentrations of Fe were expressed as percent Fe by weight. For the roots extracted with DCB, EDTA, and DTPA, dry root weights were corrected for the removal of FeOOH by the extraction technique (corrected weight = dry weight + 1.591 weight of Fe extracted; 1.591 = MW FeOOH/MW Fe). Results were analyzed using Duncan's Multiple Range Test as available on SPSS (Nie et al., 1975).

RESULTS—The data indicated that considerable Fe was present on or in the roots of *P. communis*. The total extraction detected concentrations of 8.58 ± 0.39% Fe by weight. Total Fe was also determined for each extraction procedure by summing the extracted Fe and Fe remaining in the roots. Total Fe determined by the hot DCB, cold DCB, and EDTA extraction techniques were greater than total Fe determined by the total extraction, while Fe

determined by the DTPA technique was less. Only the EDTA estimate of total Fe was statistically different from the total determination (Table 1).

There were significant differences in the extraction efficiencies among the extraction techniques utilized. The hot and cold DCB techniques extracted 98.3% and 98.6% of the total Fe (extractable Fe + remaining Fe) respectively, and were not significantly different. The EDTA technique extracted significantly less of the total Fe, 53.7%, than the DCB techniques, and the DTPA technique extracted significantly less than all the other techniques, 8.7% of the total Fe (Table 1). Concentrations of Fe remaining in washed roots was highest in the DTPA washed roots, lowest in the hot and cold DCB-washed roots, and intermediate in the EDTA washed roots.

DISCUSSION—In contrast to the DCB techniques where roots stained with FeOOH prior to the wash emerged as white intact roots, both the EDTA and DTPA techniques left considerable Fe staining on the surface of the washed roots. Both of these techniques appeared to be inefficient in extracting FeOOH. The EDTA technique left 4.32 ± 0.34% Fe by weight in (or on) washed roots while the DTPA technique left 7.32 ± 0.31% Fe by weight. Schierup and Larsen (1981) and Chiaudani (1969) found that metals, including Cu, Zn, Pb, and Cd, were preferentially accumulated by roots of *Phragmites* sp. Concentrations of these metals in roots ranged in the order of 1 to 10 times the concentrations found in leaf tissues. If the same pattern holds for Fe, then Fe concentrations in washed roots of this study (if all external Fe was extracted) should have been in the order of 1 to 10 times typical leaf Fe concentrations. Reimer and Toth (1968) analyzed the Fe content of leaves of *P. communis* and found 0.01% to 0.05% Fe by weight. Mayer and Gorham (1951) reported 0.023% Fe by weight in leaves of the same species. In this study, the concentrations of Fe in both the EDTA- and DTPA-washed roots were significantly higher than 1 to 10 times these values, ranging from roughly 100 to 700 times higher. This indicates that considerable external Fe remained on the surface of the EDTA- and DTPA-washed roots.

When compared to the EDTA and DTPA techniques, both the hot and cold DCB techniques were efficient at extracting FeOOH. In all respects, these two techniques were not significantly different. Both extracted 98% to 99% of the total Fe, leaving only 0.14 ± 0.01% and 0.12 ± 0.01% Fe by weight (respectively) in washed roots. The values for washed roots range from 2 to 14 times the leaf values reported by Reimer and Toth (1968) and Mayer and Gorham (1951), which suggests that the external Fe was efficiently extracted and the internal Fe was unaffected.

The amount of Fe extracted by the two DCB techniques was essentially the same as total Fe determined in the total extraction. It is also worth noting that while total Fe determined by both the DCB techniques was higher than total Fe determined by the total extraction, all three means were not significantly different. This might suggest that the total analysis of Fe is equally valid as an estimate of external Fe. However, this result is to be expected when up to 98% or 99% of the root Fe may be external. To compare the total Fe extraction with the DCB extractions, roots with a considerably smaller ratio of external to internal Fe must be analyzed.

Although differences between total Fe determined by the total and DCB extraction techniques were not significantly different, the DCB techniques may overestimate total Fe. We have corrected the dry weight of roots for FeOOH extracted in the washing procedure, but this may underestimate the total weight loss. Some minor loss of lateral roots in the DCB wash solution was noted and one would also expect the extraction of other ions such as Mn. Corrections for these weight losses have not been made; thus overestimation of total Fe may have resulted.

We have not evaluated the differences that different root preparation techniques may have on the proportion of Fe extracted by the various extraction techniques. Bacha and Hossner (1977) used fresh roots (prewashed in distilled water) in their analysis. This preparation most closely approximates our own procedure. Chen, Dixon and Turner (1980) used freeze-dried roots subsequently ovendried at 115 C overnight, and Mendelssohn and Postek (1982) used roots frozen over dry ice. It would seem reasonable that the first technique would result in the least disruption of intact cells, and hence should in theory be the preferred technique.

Unlike roots of *T. latifolia,* roots of *P. communis* showed little differences in structural damage as a result of washing with hot or cold DCB. However, considering the equality of the hot and cold DCB techniques demonstrated in this study, it would seem most preferable to utilize the cold extraction technique as it should minimize the extent of structural damage to more fragile roots.

LITERATURE CITED

ARMSTRONG, W. 1967. The oxidising activity of roots in waterlogged soils. Physiol. Plant. 20: 920–926.

———. 1972. A re-examination of the functional significance of aerenchyma. Physiol. Plant 27: 173–177.

———. 1978. Root aeration in the wetland condition. *In* D. E. Hook and R. M. M. Crawford [eds.], Plant life in anaerobic environments, pp. 269–297. Ann Arbor Science Publishers, Ann Arbor, Mich.

BACHA, R. E., AND L. R. HOSSNER. 1977. Characteristics of coatings formed on rice roots as affected by iron and manganese additions. Soil Sci. Soc. Amer. J. 41: 931–935.

CHIAUDANI, G. 1969. Contenuti normali ed accumuli di rame in *Phragmites communis* L. come riposta a quelli nei sedimenti di sei laghi italiani. Mem. Ist. Ital. Idrobiol. 25: 81–95.

CHEN, C. C., J. B. DIXON, AND F. T. TURNER. 1980. Iron coatings on rice roots: Mineralogy and quantity influencing factors. Soil. Sci. Soc. Amer. J. 44: 635–639.

FOY, C. D., A. L. FLEMING, AND J. W. SCHWARTZ. 1981. Differential resistance of weeping lovegrass genotypes to iron-related chlorosis. J. Plant. Nutr. 3: 537–550.

JACKSON, M. L. 1958. Soil chemical analysis. Sixth printing, Prentice Hall Inc. Published by the author, University of Wisconsin, Madison, Wisconsin.

LINDSAY, W. L., AND W. A. NORVELL. 1978. Development of a DTPA soil test for zinc, iron, manganese and copper. Soil Sci. Soc. Amer. 42: 421–428.

MAYER, A. M., AND E. GORHAM. 1951. The iron and manganese content of plants present in the natural vegetation of the English Lake district. Ann. Bot. 15: 247–263.

MENDELSSOHN, I. A., AND M. T. POSTEK. 1982. Elemental analysis of deposits on the roots of *Spartina alterniflora* Loisel. Amer. J. Bot. 69: 904–912.

NIE, N. H., C. H. HULL, J. G. JENKINS, K. STEINBRENNER, AND D. H. BENT. 1975. Statistical package for the social sciences, second ed. McGraw-Hill, Toronto.

REIMER, D. N., AND S. J. TOTH. 1968. A survey of the chemical composition of aquatic plants in New Jersey. N.J. Agric. Exp. Stn. Coll. Agric. Environ. Sci., Rutgers Univ. Bull. 820.

SCHIERUP, H-H., AND V. J. LARSEN. 1981. Macrophyte cycling of zinc, copper, lead and cadmium in the littoral zone of a polluted and a non-polluted lake. I. Availability, uptake and translocation of heavy metals in *Phragmites australis* (Cav.) Trin. Aquat. Bot. 11: 197–210.