

# High-pressure homogenisation prior to slurry introduction electrothermal atomic absorption spectrometry for metal determinations in wood pulps

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High-pressure homogenisation of softwood pulp fibres was evaluated as a sample preparation procedure prior to Cu, Fe and Mn determination by electrothermal atomic absorption spectrometry. Although homogenisation in tetramethylammonium hydroxide proved to be unsuitable, fibres that had been pre-swollen with aqueous zinc chloride were readily homogenized to result in slurries that provided repeatable estimates of analyte concentrations, which were analogous to estimates obtained from solutions prepared by conventional or microwave-assisted digestion with strong oxidants. The release of analytes into the aqueous phase however was incomplete, even with the addition of EDTA or DTPA complexing reagents to the crude fibre suspension or cellulase plus xylanase-assisted digestions of the suspension post-homogenisation. The advantages of this procedure for sample preparation included time saving and decreased operator intervention. Sample preparation was completed in 5 min.

## Introduction

Conventional sample preparation of biological materials prior to atomic spectrometry involves complete solubilisation of the analyte and matrix, which is achieved typically by oxidative mineralisation of the organic matter and solubilisation of the residue in a suitable solvent.<sup>1-4</sup> Even with microwave-assisted digestion, complete dissolution can usually be achieved by a suitable choice of digestion conditions, but complete decomposition of the organic matrix in biological/botanical samples is appreciably more difficult. Often complete mineralisation is achieved only with supplemental treatment of the digested matrix with H<sub>2</sub>O<sub>2</sub> or even HF.<sup>5</sup> These digestion procedures can be labour intensive, time consuming and prone to contamination errors. In consequence, there is a continuing interest in the development of simplified sample preparation techniques. The preparation/introduction of slurried samples continues to attract considerable attention because of the ease with which quasi-stable preparations can be generated and their compatibility with conventional liquid handling techniques. Within the general field of solid sampling analysis, it is the use of slurried samples<sup>6-11</sup> that has become the most popular approach to trace element determination.

Dispersions, with a tendency to rapidly segment, have been reproducibly sampled by using ultrasonic agitation,<sup>12,13</sup> air or argon<sup>14</sup> bubbling, vortex mixing, or magnetic stirring.<sup>15</sup> Partial digestion procedures to produce carbonaceous slurries have also been successfully applied to the analysis, by ICP-AES, of a series of standard reference materials of biological origin.<sup>16</sup> A variety of alkylammonium hydroxide formulations have been used extensively to solubilise tissue.<sup>17-20</sup> A further development has involved the use of high pressure to generate quasi-stable slurries prior to electrothermal atomic absorption spectrometry (ETAAS).<sup>21-24</sup>

In TMP (thermomechanical pulp) processing,<sup>25</sup> chips of softwoods destined for paper products are pre-steamed at > 100 °C and then refined mechanically in a primary stage at an elevated temperature and pressure, followed by a secondary

stage of pressurised refining at ambient temperature. The product is suitable for printing (typically newsprint). In the Kraft pulping process, the wood chips are cooked in a solution of NaOH and Na<sub>2</sub>S to soften/solubilise the lignin that binds the individual fibres together.<sup>26</sup> Depending on the subsequent processing, a variety of products can be prepared from Kraft pulps ranging from packaging materials to white papers.

Heavy metals are natural constituents of the wood fibres,<sup>27-29</sup> although the quantities present vary according to the species of tree and the geographic locale of its growth. Interest in the metal profiles in pulps has increased in recent years because of the adverse influence on processing with totally chlorine-free (TCF) bleaching techniques. Peroxide is used extensively as a cost-effective bleaching agent in chemical pulping as well as a brightening agent in mechanical pulping. Troublesome metal ions, particularly copper, iron and manganese, accelerate the decomposition of peroxide/ozone in alkaline media<sup>30,31</sup> resulting in the formation of superoxide anions and hydroxyl radicals that lower the pulp brightness by reducing the effective quantity of the bleaching chemical. Additionally, these decomposition products can react with lignin to form coloured products that darken the pulp and that can mediate the degradation of cellulose, causing chain breaks in the polymer structure resulting in decreased strength in the finished paper product. In consequence, the availability of heavy metals is controlled by complexation<sup>32-34</sup> with EDTA (disodium ethylenediaminetetraacetate) or DTPA (disodium diethylenetriaminepentaacetate) followed by a water wash to remove solubilised complexes from the pulp slurry prior to bleaching/brightening. To minimise the consumption of chelating chemical(s), metal profiles in pulps are monitored routinely.

A variety of organic and ionic salt solutions have been used to swell, suspend, or dissolve cellulose materials. As a guide to the relative swelling capabilities of various cations and anions, the lyotropic series<sup>35</sup> can be consulted. Treatment with aqueous ZnCl<sub>2</sub> has been reported<sup>36</sup> to decrease the degree of polymerisation and crystallinity of cellulose fibres resulting in

appreciable reductions in fibre length. The objective of the current report is to devise a simple and rapid method to determine the content of Cu, Fe and Mn in softwood pulps

## Experimental

### Reagents

Dimethyl sulfoxide (DMSO), disodium ethylenediaminetetraacetate (EDTA), lithium chloride, *N,N*-dimethylacetamide, 4-methylmorpholine-*N*-oxide (MMNO), tetramethylammonium hydroxide (TMAH), tris(hydroxymethyl)aminomethane (THAM) and ZnCl<sub>2</sub> were purchased from Sigma-Aldrich (Oakville, ON, USA). Disodium diethylenetriaminepentaacetate (DTPA) was purchased from Acros Chemicals (Morris Plains, NJ, USA) and cellulase (Celluclast 1.5 L) was purchased from Novo Nordisk Biochem (Philadelphia, PA, USA). Xylanase was generously donated by Dr. D. S. Argyropoulos, Pulp and Paper Research Institute, McGill University. Both enzyme preparations were liquid solutions. Aqueous Cu, Fe and Mn solutions (1 000 µg mL<sup>-1</sup>, traceable to NIST primary standard), were purchased from SCP Chemical Co. (St-Laurent, QC, Canada). Nitric acid, environmental grade, was purchased from Alfa Aesar (Ward Hill, NJ, USA). Distilled deionised water (resistivity 18.2 MΩ cm<sup>-1</sup>), generated with a Milli-Q purification system (Millipore, Bedford, MA, USA), was used throughout.

### Samples

Air dried samples of balsam fir, black spruce and jack pine as well as thermomechanical pulp and Kraft pulp were kindly provided by Dr Cyril Heitner and Dr J. Ing, Paprican, Pointe Claire, QC, Canada.

### Sample preparation

Pulps were ground to pass a 0.5 mm screen, in a Tecator Cyclotec sample mill (Tecator AB, Höganäs, Sweden) and then re-ground to pass through a 250 µm aperture sieve with a Wiley cutting mill. An accurately weighed sample (approximately 0.1 g) was wetted with 5 mL water then swollen with 2 mL of ZnCl<sub>2</sub> solution (65% w/v). After equilibration for 1–2 min, the suspension was diluted to 50 mL with ethanol–water (1 + 9, v/v). The resulting suspension was processed through the homogeniser (Emulsiflex Model C5) operated in the recycle mode, while the working pressure gradually increased from 20 to 80 psi, and then processed for a further 3 min (equivalent to 5 sequential passes) while maintaining the air pressure at 80 psi. Alternatively, a weighed, ground sample was suspended in 50 mL of ethanol–water (1 + 9 v/v) containing 0.25% (m/v) TMAH prior to homogenisation. Alternatively, a weighed, dried and ground sample was swollen in 10 mL DMSO or *N,N*-dimethylacetamide containing 0.1 g LiCl or 10 mL of 1% (v/v) aqueous MMNO.

### Homogeniser

The Model C5 (Avistin Inc., Ottawa, ON) was modified by: replacing the stainless-steel (ss) balls within the inlet and outlet check valve assemblies with ceramic balls; removing the shims from the spring assembly; and replacing the variable-strength springs (0.4–0.6 kg) with equal strength springs (0.4 kg). The ss components (with the exception of the chrome-plated plunger) of the disassembled homogeniser were soaked in 2% NaOH (w/w) containing 25% Na gluconate (w/w) at 70 °C during 1 h, rinsed with distilled water and soaked in 25% HNO<sub>3</sub> at 70 °C for 1 h. Finally, the assembled homogeniser was rinsed with distilled H<sub>2</sub>O until pH and conductivity measurements in the outflow were similar to the inflow.

### Block digestion

Acid digested samples were prepared following the procedure described by the Technical Association of Pulp and Paper Industries (TAPPI) standard methods (1996–97). An accurately weighed, dried, ground sample (approximately 2 g) was added to a digestion tube followed by 10 mL HNO<sub>3</sub> (63% v/v). The sample was refluxed at 130 °C for 1 h then permitted to cool. Subsequently, 3 mL H<sub>2</sub>O<sub>2</sub> was added and the digestion was continued at 130 °C for a further 1 h. On cooling, the resulting clear digest was diluted to 100 mL with distilled deionised water.

### Microwave digestion

HNO<sub>3</sub> (9 mL, 63% v/v) was combined with approximately 0.1 g of an accurately weighed, dried, ground sample in a Teflon PVA digestion vessel. When the reaction became less vigorous, 1 mL H<sub>2</sub>O<sub>2</sub> was added and the vessel was sealed using the capping station of the Microwave Laboratory System (Milestone, Monroe, CT, USA). The vessel was transferred to the microwave cavity of the instrument, heated gradually (power increase to 1000 W over 5 min) and digested at this setting for a further 15 min. After cooling to ambient temperature, the vessel was vented and then opened. The resulting digest was transferred to a 50 mL volumetric flask and diluted to the mark with distilled deionised water.

### ETAAS

Copper, iron and manganese determinations were performed using a hot injection technique on a Varian Model 300 GFAAS system equipped with an autosampler, pyrolytically coated platform graphite tubes, conventional hollow cathode lamps and Zeeman-effect background correction. Ashing–atomisation curves were generated for the standard in the presence/absence of co-injected pulp digest. In the presence of the palladium–citric acid modifier<sup>21,22</sup> no loss in the Fe or Mn signal was observed at an ashing temperature of ≤2000 °C. Analytical operating parameters are presented in Table 1.

### Calibration

ETAAS quantification was performed by both the method of external standards (ES) and by standard additions (SA). ES, consisting of an appropriately diluted processed reagent blank and up to four levels of standard, were prepared automatically by the sample introduction device. Background corrected peak area response, resulting from three replicate injections of each diluted standard, was used to define the best fit regression equation. For SA calibrations, 10 µL aliquots of processed fluid were amended with 2, 5, or 10 µL of aqueous standard, chosen to result in a range of peak areas including signals that were one-half and at least twice the signal for the unamended sample. The data were modelled by least squares linear regression. Quantification was performed by dividing the *Y*-intercept of the regression equation by the slope of that equation and the overall standard error of estimate (SE<sub>est</sub>) was calculated from:

$$SE_{est} = (SE_{y-intercept}^2 + SE_{slope}^2)^{1/2}$$

## Results and discussion

The lack of a suitable reference material that mimics softwood pulps required that estimates of metal concentrations in this matrix be compared with results generated with an accepted analytical procedure. Conventionally, metal concentrations in softwood pulp fibres have been determined in solutions prepared by a gentle reflux in nitric acid–H<sub>2</sub>O<sub>2</sub>. The block

**Table 1** Graphite furnace operating parameters for the determination of Cu, Fe or Mn

	Copper	Iron	Manganese
Wavelength/nm	324.7	386.0	279.5
Lamp current/A	12	12	8
Slit width/mm	0.1	0.2	0.2
Injection $T/^{\circ}\text{C}$	60	60	60
Pre-injection	No	Yes	No
Last dry step (5 s)/ $^{\circ}\text{C}$	No	250	250
Charring sequence	Ar purge gas (3 L min <sup>-1</sup> ) on. 5 s ramp to 900 $^{\circ}\text{C}$ , 20 s hold	Ar purge gas (3 L min <sup>-1</sup> ) on. 10 s ramp to 1100 $^{\circ}\text{C}$ , 21 s hold	Ar purge gas (3 L min <sup>-1</sup> ) on. 10 s ramp to 1200 $^{\circ}\text{C}$ , 20 s hold
Cool down	None	None	None
Atomisation	1.0 s ramp to 2300 $^{\circ}\text{C}$ , Ar off, 2 s hold	1.2 s ramp to 2400 $^{\circ}\text{C}$ , Ar off, 4 s hold	0.7 s ramp to 2200 $^{\circ}\text{C}$ , Ar off, 2 s hold
Measurement time	3 s	6 s	3 s
Sample modifier	5 $\mu\text{L}$ of 1% (m/m) $\text{NH}_4\text{NO}_3$ for 10 $\mu\text{L}$ sample	5 $\mu\text{L}$ of 500 mg L <sup>-1</sup> Pd +2.5% citric acid for 10 $\mu\text{L}$ sample	5 $\mu\text{L}$ of 500 mg L <sup>-1</sup> Pd +2.5% citric acid for 10 $\mu\text{L}$ sample

digestion was completed in approximately 2.5 h. In an effort to decrease the sample processing time, microwave-assisted digestion was also evaluated. The results of replicate determinations are recorded in Table 2. Microwave-assisted digestions were completed in approximately 30 min.

The modifications to the homogenising instrument and subsequent passivation of the components reduced the magnitude of, but did not entirely eliminate, the signal from the solvent post-processing through the homogeniser (Table 3). In practice, the assembly was washed four successive times with solvent prior to processing a sample and measured levels of Cu, Fe and Mn were corrected for the mean levels in the processed solvent.

Concentrations of Cu, Fe and Mn in pulp slurries prepared by high-pressure homogenisation (HPH) in tetramethylammonium hydroxide (TMAH) solvent were characterised by unacceptably high relative standard deviations (RSDs) among replicate determinations when compared with RSDs observed for strong acid digests for the same pulps. Moreover, the homogenising instrument was prone to partial blockage as fibres accumulated around the springs of the check valve assemblies. In consequence, other solvents were evaluated for their ability to release the analyte metals from the pulp matrix. Among the five chemicals that were evaluated (4-methylmorpholine-*N*-oxide, DMSO, *N,N*-dimethylacetamide, LiCl and  $\text{ZnCl}_2$ ), zinc chloride proved to be an efficient swelling agent for the softwood pulps.

In preliminary trials, calibrations were performed by both the method of external standards and the method of standard additions. The slopes of the calibration plots, generated by either method, were virtually identical to each other and the pyrolysis-atomisation curves for standards in the presence/absence of slurried pulp were virtually identical to each other. Thus, there was no evidence for matrix effects in this medium so that subsequent analyses were performed with external standards calibration. The five pulps samples that were investigated for their contents of Cu, Fe and Mn included a balsam fir, a black spruce, a jack pine sample, a mixture of black spruce and balsam fir that had been generated by

**Table 3** Analyte concentrations (pg per 5  $\mu\text{L}$ ) in successive fractions of solvent [distilled water or water-ethanol (9 + 1 v/v) containing 0.25% TMAH] post processing through the modified homogenizing instrument

Solvent	Copper	Iron	Manganese
$\text{H}_2\text{O}$	1.2	20.0	ND <sup>a</sup>
$\text{H}_2\text{O}$	1.0	20.1	ND
$\text{H}_2\text{O}$	0.56	18.6	ND
$\text{H}_2\text{O}$	0.50	18.2	ND
TMAH	5.6	29.7	5.0
TMAH	5.2	30.0	2.0
TMAH	5.1	34.0	2.0
TMAH	3.4	32.3	2.0

<sup>a</sup>ND = none detected, limit of detection (LOD) = 0.4 pg Mn  $\mu\text{L}^{-1}$ .

thermomechanical treatment (TMP processing) and a sample that had been prepared chemically (Kraft processing). The results for total slurry sampling and acid digestion are recorded in Table 4. No significant differences in the mean metal content were observed between HPH-treated and acid-digested (AD) sub-samples. The degree of discord between the two preparation procedures [(homogenized-acid digested)/(acid digested)] was acceptably low and randomly distributed among the 18 metal determinations. The RSDs for metal determinations in individual slurries prepared from  $\text{ZnCl}_2$ -swollen pulps were acceptably low in all cases and not appreciably different from the RSDs observed for metal determinations in acid digests. In short, the two sample preparation procedures provided analogous estimates of the metal concentrations. A simple mixing of the homogenate with a Pasteur pipette, prior to sample introduction into the ETAAS instrument, was sufficient to achieve good repeatabilities.

#### Analyte release to the supernatant phase

If the analyte is transferred to the supernatant phase during processing, matrix effects can be diminished/eliminated and the useful lifetime of the graphite tube is extended by the fact that

**Table 2** Mean Cu, Fe and Mn concentrations ( $\mu\text{g g}^{-1} \pm 1$  relative standard deviation based on three replicate samples) in wood pulp that had been processed with strong oxidants by either block digestion or by microwave-assisted digestion

Pulp origin/treatment	Cu		Fe		Mn	
	Block ( $n = 5$ )	Microwave ( $n = 3$ )	Block ( $n = 5$ )	Microwave ( $n = 3$ )	Block ( $n = 5$ )	Microwave ( $n = 3$ )
Black spruce	$4.0 \pm 1$	$4.5 \pm 5$	$32.6 \pm 3$	$37.2 \pm 2$	$38.9 \pm 1$	$35.9 \pm 2$
Thermomechanical pulp	$3.6 \pm 3$	$3.3 \pm 6$	$19.3 \pm 4$	$21.6 \pm 4$	$6.5 \pm 2$	$6.1 \pm 2$
Kraft pulp	$4.4 \pm 3$	$4.6 \pm 6$	$15.7 \pm 2$	$16.1 \pm 5$	$64.0 \pm 1$	$57.9 \pm 2$

**Table 4** Mean Cu, Fe and Mn concentrations<sup>a</sup> in ZnCl<sub>2</sub>-solubilised wood pulps processed by high-pressure homogenization (HPH) or by acid digestion (AD) then subjected to metals determination by ETAAS

Pulp source	Copper			Iron			Manganese		
	HPH <sup>b</sup>	AD <sup>c</sup>	Discord (%) <sup>d</sup>	HPH <sup>b</sup>	AD <sup>c</sup>	Discord (%) <sup>d</sup>	HPH <sup>b</sup>	AD <sup>c</sup>	Discord (%) <sup>d</sup>
Black spruce #1 <sup>e</sup> ( <i>n</i> = 5)	3.5 ± 5	4.0 ± 1	-12	36.0 ± 6	32.6 ± 3	10	38.5 ± 4	38.9 ± 1	-1
Black spruce #2 <sup>e</sup> ( <i>n</i> = 5)	3.5 ± 3	3.9 ± 2	-9	37.2 ± 3	34.9 ± 5	7	38.2 ± 9	38.7 ± 1	-1
Jack pine ( <i>n</i> = 3)	2.9 ± 3	2.6 ± 2	12	26.3 ± 5	25.8 ± 3	2	39.7 ± 2	39.4 ± 4	1
Balsam fir ( <i>n</i> = 3)	1.8 ± 4	1.5 ± 2	16	43.1 ± 7	41.6 ± 3	4	62.3 ± 3	60.7 ± 1	3
TM pulp ( <i>n</i> = 3)	3.4 ± 5	3.6 ± 3	-4	21.8 ± 3	19.3 ± 4	12	6.6 ± 2	6.5 ± 5	1
Kraft pulp ( <i>n</i> = 3)	4.7 ± 6	4.4 ± 3	8	15.0 ± 5	15.7 ± 2	-5	65.6 ± 1	64.0 ± 1	3

<sup>a</sup>µg g<sup>-1</sup> ± 1 relative standard deviation based on *n* replicate analyses performed on different days. <sup>b</sup>High-pressure homogenized dispersion. <sup>c</sup>Acid digested solution. <sup>d</sup>Degree of discord [(homogenized - acid digested)/(acid digested)]. <sup>e</sup>Separately-milled sub-samples from the same sample matrix.

there is less total material actually transferred and less residue accumulated in the tube post atomisation. Previous studies had demonstrated that Cd, Cu and Pb in frozen cervine tissue and in animal feeds of zoological origin were transferred quantitatively to the supernatant phase during HPH processing in TMAH solvent.<sup>21,22</sup> By contrast, recoveries of selenium in the supernatant phase from the HPH of certified reference materials (CRMs) of zoological origin, and from wheat flour or animal feeds of botanical origin, were accomplished only with enzymatic digestion of the homogenate,<sup>23</sup> or by multiple passes through the instrument.<sup>24</sup> The apparent levels of analyte in the supernatant fraction from softwood pulp slurries that had been prepared by high pressure homogenisation (HPH) were consistently lower than in acid digests prepared from the same matrix (Table 5). Moreover, incomplete transfer was also observed for extended processing times and for higher homogeniser operating pressures. A further complication was evidenced when HPH slurries were stored for up to 10 days at 4 °C (Table 6). Whereas the Mn was released into the supernatant fraction quantitatively and remained with this fraction during 10 days of storage, the copper and iron were released from the solids only partially by homogenisation, and, with extended storage, the iron became re-adsorbed to the solids fraction.

An alternate procedure for slurry preparation involved the addition of a chelating reagent to the HPH-generated pulp suspension. Chelating reagent (50 mL of 0.1–0.5% w/v EDTA or DTPA) was added to the ethanol–water pulp suspension that had been pre-swollen with ZnCl<sub>2</sub>, processed through the homogeniser and then acidified to pH, 3.5, 4.5 or 5.5 with HCl.

**Table 5** Percent recovery of Cu, Fe or Mn in the supernatant fraction from slurries prepared by high pressure homogenization

Pulp source	Copper	Iron	Manganese
Black spruce	45	67	91
Jack pine	39	65	90
Balsam fir	45	70	94
Thermomechanical pulp	36	52	82
Kraft pulp	45	64	88

The amended suspension was then heated to 70 °C, with constant stirring, for 20 min and assayed for heavy metals. In preliminary ranging trials, the extraction of metals was increased for concentrations of chelant from 0.1–0.3%, but further increases to 0.5% caused only minimal changes in recoveries in the supernatant phase.

The results of the processing of replicate samples are summarised in Table 7. Whereas the Fe and Mn burdens were transferred virtually quantitatively to the supernatant phase, a portion of the copper remained with the solids. Efforts were also made to release analytes by performing an enzymatic digestion on the crude homogenates (Table 8). A mixture of xylanase and cellulase was incubated with the slurry for 3 h at 80 °C. Preliminary ranging trials indicated that 1 mL of cellulase reagent and 0.5 mL of xylanase reagent resulted in a maximum release of analyte metals into the supernatant phase. In a subsequent trial, the cellulase–xylanase enzyme mix was added to the pulp suspension prior to or post HPH processing. After 3 h of incubation at 80 °C (subsequent to HPH), the resulting digests were assayed for their content of D-glucose (as a measure of cellulolytic activity). There was an increased content of reducing sugars in all pulp mixtures that had been amended with the enzyme mixture, then homogenised and incubated (relative to incubation followed by homogenization). A possible explanation for the increased enzyme activity, post-homogenisation, is the partial rupture of cell wall material and the reduction in particle size that would increase the area of exposed surfaces to the enzyme. This protocol was adopted for subsequent trials. Three pulps were then incubated with the enzyme mixture (3 h at 80 °C) post homogenization. The results of metal determinations in the total slurry and in the supernatant fraction are recorded in Table 8. Whereas the Fe and the Mn contents in the pulp were released quantitatively, the Cu content was released efficiently from the three pulps (86 ± 7%) but not quantitatively relative to strong acid digestion.

Transition metals can be chelated by carbonyl and/or carboxylate functional groups within the cellulose/hemicellulose/lignin polymers of the pulp. Of the three metal ions, Cu and Fe are considered to be more tightly bound to the fibres although

**Table 6** Variations, with time, of the mean analyte concentration<sup>a</sup> in the total slurry or in the supernatant fraction

Source	Analyte	0 days		6 days		10 days	
		TSS <sup>b</sup>	S- fraction <sup>c</sup>	TSS	S- fraction	TSS	S- fraction
TM pulp	Cu	3.4 ± 5	1.3 ± 2	3.2 ± 4	1.1 ± 2	3.3 ± 4	0.97 ± 13
	Fe	20.2 ± 4	11.3 ± 6	19.1 ± 6	ND <sup>d</sup>	19.3 ± 4	ND
	Mn	6.6 ± 3	6.4 ± 5	6.7 ± 3	5.9 ± 5	6.5 ± 5	5.8 ± 1
Kraft pulp	Cu	4.3 ± 8	1.9 ± 3	4.0 ± 4	1.6 ± 4	3.9 ± 1	1.3 ± 8
	Fe	14.6 ± 3	9.0 ± 3	14.8 ± 9	ND	12.9 ± 5	ND
	Mn	63.9 ± 2	62.3 ± 4	63.6 ± 3	63.6 ± 2	63.5 ± 3	58.1 ± 4

<sup>a</sup>µg g<sup>-1</sup> ± 1 relative standard deviation based on three replicate determinations. <sup>b</sup>Total slurry sampling. <sup>c</sup>Supernatant fraction sampling. <sup>d</sup>ND = none detected, limit of detection (LOD), less than 6 pg Fe µL<sup>-1</sup>.

**Table 7** Mean concentration<sup>a</sup> of Cu, Fe or Mn in the supernatant fraction from pulps that had subjected to high pressure homogenisation, post-chelation treatment with EDTA<sup>b</sup> or DTPA<sup>c</sup> at pH, 3.5, 4.5 or 5.5

Sample	Analyte	Block digestion	EDTA			DTPA		
Black spruce	pH		5.5	4.5	3.5	5.5	4.5	3.5
	Cu	3.8 ± 5	1.7 ± 13	2.2 ± 5	2.4 ± 5	1.9 ± 10	2.6 ± 1	2.7 ± 4
	Fe	36.1 ± 6	34.4 ± 5	35.3 ± 5	30.7 ± 2	35.8 ± 5	35.8 ± 5	31.6 ± 3
Kraft pulp	Mn	38.7 ± 3	36.8 ± 2	36.6 ± 3	35.9 ± 1	38.4 ± 1	37.9 ± 0	37.7 ± 2
	Cu	4.3 ± 4	2.1 ± 3	2.3 ± 5	2.8 ± 2	3.1 ± 2	2.6 ± 7	2.5 ± 3
	Fe	16.3 ± 4	15.1 ± 7	15.9 ± 2	14.3 ± 3	15.3 ± 9	16.8 ± 4	14.5 ± 7
TM pulp	Mn	65.6 ± 0	62.9 ± 1	62.7 ± 2	61.9 ± 3	65.2 ± 2	65.3 ± 3	62.7 ± 1
	Cu	3.2 ± 2	1.4 ± 11	1.8 ± 7	2.5 ± 5	1.7 ± 4	2.0 ± 8	2.5 ± 5
	Fe	20.7 ± 8	18.1 ± 4	19.9 ± 6	17.4 ± 6	19.7 ± 10	20.5 ± 4	17.6 ± 5
	Mn	6.5 ± 3	6.2 ± 3	6.3 ± 5	6.2 ± 1	6.4 ± 3	6.5 ± 0	6.3 ± 2

<sup>a</sup>Mean concentration ± 1 relative standard deviation based on three replicate determinations. <sup>b</sup>Disodium ethylenediaminetetraacetate. <sup>c</sup>Disodium triethylenediaminepentaacetate.

**Table 8** Mean concentrations<sup>a</sup> of Cu, Fe or Mn in acid- or enzyme-digested pulp slurry that had been homogenized at high pressure

Pulp source	Analyte	Acid-digested	Enzyme-digested		
			TSS <sup>b</sup>	S-fraction <sup>c</sup>	% Recovery in the supernatant <sup>d</sup>
TM pulp (n = 3)	Cu	3.5 ± 3	3.2 ± 5	2.8 ± 3	88 ± 5
	Fe	19.7 ± 4	20.1 ± 6	19.5 ± 3	97 ± 7
	Mn	6.5 ± 2	6.6 ± 3	6.6 ± 0	100 ± 3
Jack pine (n = 3)	Cu	2.9 ± 3	2.7 ± 5	2.2 ± 7	82 ± 10
	Fe	26.7 ± 3	26.5 ± 10	25.1 ± 7	95 ± 5
	Mn	39.9 ± 2	39.7 ± 1	39.1 ± 0	99 ± 8
Kraft pulp (n = 3)	Cu	4.3 ± 3	4.6 ± 3	3.9 ± 2	87 ± 7
	Fe	15.1 ± 5	15.7 ± 6	14.4 ± 3	92 ± 9
	Mn	65.8 ± 1	65.7 ± 3	65.4 ± 1	100 ± 2

<sup>a</sup>Mean concentration (µg g<sup>-1</sup>) ± 1 relative standard deviation based on n different samples. <sup>b</sup>Total slurry sampling of the enzyme digest. <sup>c</sup>Supernatant fraction from the enzymatic digest. <sup>d</sup>Percent of analyte released to the supernatant fraction.

the actual mechanism of binding is still not known with certainty.

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