

## X-ray Microanalysis of Normal Primary Tooth Dentine and Primary Tooth Dentine in Teeth Diagnosed Dentinogenesis Imperfecta Type II

Noren JG<sup>1\*</sup>, Sabel N<sup>1</sup> and Cornell DH<sup>2</sup>

<sup>1</sup>Professor Emeritus, Department of Pediatric Dentistry, Institute of Odontology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

<sup>2</sup>Department of Earth Sciences, University of Gothenburg, Gothenburg, Sweden

### \*Corresponding author

Jorgen G Noren, Professor Emeritus, Department of Pediatric Dentistry, Institute of Odontology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, Tel: +46707494420; E-mail: jorgen.noren@odontologi.gu.se

Submitted: 19 Sep 2018; Accepted: 25 Sep 2018; Published: 03 Oct 2018

### Abstract

**Background:** Dentinogenesis Imperfecta (DI) is an inherited single-gene disorder affecting the dentine. The mineral concentration in DI type II dentine has been shown to be significantly lower compared to what was found in normal dentine, however, reports of elemental analyses of the dentine in Dentinogenesis Imperfecta are few.

**Aim:** The aim of this study was to analyse the elemental composition of dentine in primary teeth from children with DI and from normal sound primary teeth using X-ray microanalysis.

**Materials and Methods:** X-ray microanalysis of the elements C, O, Na, Mg, P, Cl, K and Ca was performed in the dentine of five normal primary teeth and in seven primary teeth diagnosed DI. The analysis was made in a low magnification in 10 points from the enamel-dentine junction/root surface toward the pulp. The data was also evaluated with an inductive analysis.

**Results:** Lower values for C were found in DI-dentine compared with normal dentine. Na had significantly higher values in DI-dentine while Mg had significantly lower values. The inductive analysis revealed that Na and Mg were the most important elements for discriminating DI-dentine from normal dentine.

**Conclusion:** Dentine in primary teeth from patients diagnosed with Dentinogenesis Imperfecta type II analysed with XRMA have lower values of C and Mg and higher values of O and Na compared with normal primary dentine.

**Keywords:** Dentinogenesis Imperfecta, Elemental composition, X-ray microanalysis

### Abbreviations

D: Dentine

DI: Dentinogenesis imperfecta

E: Enamel

EDJ/RS: Enamel-dentine junction/Root surface

N: Normal

PD: Pulpal dentine area

SEM: Scanning electron microscopy

XRMA: X-ray microanalysis

### Introduction

Dentinogenesis Imperfecta (DI) is an inherited single-gene disorder affecting the dentine which is classified into two main groups, DI and Dentin Dysplasia (DD) [1,2]. Differences in the pathological features are described, however, they are proposed to reflect variations in severity [3-6]. In several papers the genetics, clinical appearance and molecular aspects have been reported [5-10].

Clinically teeth affected by DI have an opalescent appearance with a colour varying from greyish to brown with blue streaks and often enamel is chipped off at the enamel-dentine junction [1,11-13]. Since the enamel is normal regarding its structure and mineralization the colour of the teeth is a result of the colour of the underlying dentine. In Dentinogenesis Imperfecta type II, X-ray radiographs reveal pulp chambers which progressively obliterate, resulting in a denser dentine structure enhancing the deviation of colour of the teeth. The obliterated dentine is irregular and atypical compared with normal dentine [13,14]. The defect dentine in DI type II has been attributed to the weakness in the attachment between enamel and dentine creating the problems of chipping off of the enamel at the enamel-dentine junction [15,16].

In histo-morphological studies of the dentine in primary teeth with DI Type II vascular inclusions have been described [17,18]. X-ray microtomography (XMT) has shown tubular structures in the dentine coinciding with the path of normal dentinal tubules but not continuous tubules [19]. The dysfunctional mineralisation of the dentin and obliteration of the pulp evidently leaves blood vessels in the dentin which have been tied off and in the un-decalcified

sections appear as vacuoles. The mineral concentration in DI type II dentine has been shown to be significantly lower compared to what was found in normal dentine and with needle-like crystallites indicating lack of intrafibrillar mineral [20].

Reports of elemental analyses of the dentine in Dentinogenesis Imperfecta are few and therefore the aim of this study was to analyse the elemental composition of dentine in primary teeth from children with DI and from normal sound primary teeth using X-ray microanalysis (XRMA) [21,22].

## Material and Methods

### Tooth Material

The tooth material comprised of 7 exfoliated and extracted primary teeth (1 mandibular incisor; 1 maxillary canine, 5 mandibular molar roots) from 4 patients diagnosed with Dentinogenesis Imperfecta Type II. As reference teeth served 5 exfoliated primary incisors from 5 individuals with no known medical history. The teeth were collected through the years at the department of Pediatric Dentistry, Institute of Odontology at the Sahlgrenska Academy, Göteborg, Sweden. The exfoliated and extracted teeth were collected when the children were between 7 and 10 years of age, however, the exact dates are not known. The teeth were originally stored under cold conditions in 10% neutral buffered formaldehyde and transferred to 70% ethanol before embedding.

Prior to the XRMA analysis or embedding macro photos of the teeth were taken in a Leica M80 stereo microscope (Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany) using a Leica digital camera (Leica DFC420 C, Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany) with Leica Application Suite LAS V3.7.0 (Leica Microsystems AG, Heerbrugg, Switzerland).

### Ethical Considerations

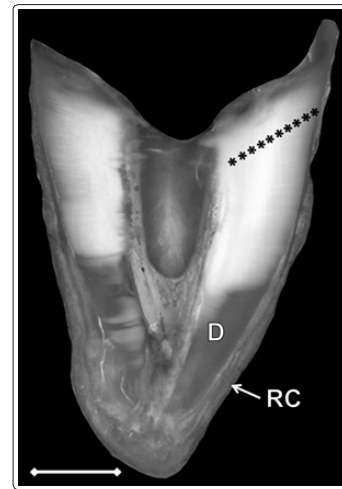
All teeth were donated by free will of the patient and with consent of the accompanying parent/parents. The collected teeth were coded to assure that teeth from the same patient were stored together; none of the teeth could be identified to any specific patient.

### Embedding and Cutting of Teeth

The teeth were oriented for sagittal cutting in bucco-lingual direction and embedded in an epoxy-resin (Epofix®, Electron Microscopy Sciences, Fort Washington, PA, USA) using embedding moulds with a diameter of 15 mm. Each tooth was cut into two halves in a Leica SP1600 Saw Microtome (Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany). The cut surface of the specimens was polished with polishing paper (grit 1200, 2400 and 4000) under water cooling. Prior to any analyses the surface was cleaned in an ultra-sonic bath in de-ionized water for 30 seconds.

### Light Microscopy

Overviews of cut and polished specimens were taken in a Leica M80 stereo microscope (Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany) at low magnification (0.75x) in incident light using a Leica digital camera (Leica DFC420) with Leica Application Suite LAS V3.7.0. (Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany). The overviews were used for orientation for the X-ray microanalysis (Figure 1).



**Figure 1:** Un-decalcified specimen of a primary tooth from a patient diagnosed with Dentinogenesis Imperfecta type II with polished surface prepared for XRMA analysis. The principle for the locations of XRMA measurements are shown as black stars in which analysis in the 10 points was performed (D=dentine; RC=root cementum; White bar=2 mm)

### X-ray Microanalyses

The polished samples were coated with gold in a plasma coater with a thickness of  $\approx 25$  nm. The XRMA analysis was performed in a Hitachi VP-SEM S-3400N (Hitachi, Tokyo, Japan) equipped with an Oxford EDS system and INCA Energy software (Oxford Instruments, Abingdon, UK). All analyses were carried out at 20 kV accelerating voltage and the working distance from sample to electron optical column was 9.6 mm, with a tolerance of  $\pm 0.1$  mm. The beam was aligned in “Microscope setup” using “Wave” in INCA when the microscope was positioned at a Faraday cage in the specimen holder. The beam current was adjusted to 6.0 nA  $\pm 0.1$  nA, and was then checked every second hour.

The session calibration was performed using cobalt metal in the specimen holder in “Energy” setting of INCA and was made after acquiring a spectrum with a live time of 40 seconds. The Oxford EDS spectrometer was calibrated for each element at regular intervals using pure metal and simple oxide standards linked to the energy and peak area calibrations of cobalt and checked using Smithsonian Institute mineral standards.

For the elemental analysis of C, O, Na, Mg, P, Cl, K and Ca, the “All elements” and “Normalized” option in the INCA software was used and a spectrum was acquired with a live time of 100 seconds. The measurements were carried out in a low magnification ( $\times 45$ ). After defining a line parallel to the dentinal tubules, the acquisition of data was automatically performed in 10 points from the enamel-dentine junction/root surface toward the pulp (Figure 1). In the teeth which were completely obliterated the 10th point was located in the middle of the section.

The number of line measurements and total number of measurements in the dentine of the five normal sound primary teeth and in the seven primary teeth diagnosed with Dentinogenesis Imperfecta type II are shown in Table 1.

**Table 1:** Number of line measurements (No. L.) and total number of measurements (Tot. no. M.) in the dentine of 5 normal sound primary teeth (N-01 – N-05) and in 7 primary teeth from patients diagnosed with Dentinogenesis Imperfecta type II (DI-01 – DI-07)

Sample	No. L.	Tot. no. M.
<b>Normal</b>		
N-01	4	40
N-02	2	20
N-03	4	40
N-04	2	20
N-05	4	40
<b>DI</b>		
DI-01	4	40
DI-02	6	60
DI-03	4	40
DI-04	5	50
DI-05	2	20
DI-06	3	30
DI-07	2	20

### Statistical Analyses

The statistical analyses were performed using the IBM SPSS Statistics for Windows, Version 21.0. (IBM Corp, Armonk, NY, USA) employing the non-parametric Mann-Whitney U Test for independent samples for each point of location for the XRMA measurements. The level of significance was set to  $p < 0.05$ .

### Inductive Analysis

An inductive analysis was performed to elucidate any relationship between the elements measured with XRMA and their origin, normal dentine or DI-dentine. All data was compiled in an Excel spread sheet, where the values (*numerical*) for the different elements (*attributes*) were set in columns, each row representing one point of measurement – one example. As *outcome*, a column of values (*discrete*) representing measurements from DI or normal dentine was added in order to enable recognition of any relationship between the different elements and the outcome values. The data was imported to the inductive analysis program Xpert Rule Analyser (Attar Software, Lancashire, UK). Before the analysis was performed, the discrete attribute was set as outcome. The results are presented in a hierarchic diagram (*knowledge tree*) in which the importance of every attribute in the inductive analysis is specified by its position in the knowledge tree. The higher in the tree, the more important for the outcome, and thus, the tree shows how different attributes affect the outcome.

An inductive analysis was performed using values for C, O, Na, Mg, P, Cl, K and Ca from the XRMA analysis with “normal dentine” and “DI-dentine” as discrete outcome values. In the analysis, 50%

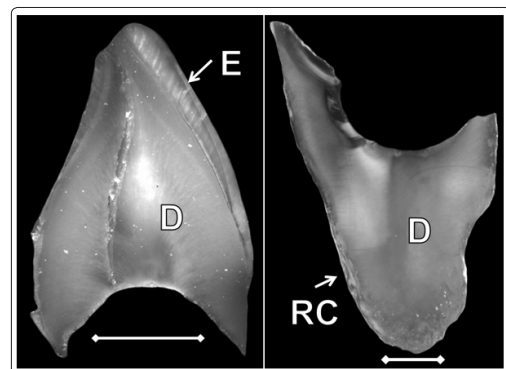
of the examples were randomly selected by the program for use in the induction of a knowledge tree (*training set*), the remaining examples were used for verification of the generated rules (*test set*). In the Verify option of the Analyser, a table will show the accuracy of the rules induced in the knowledge tree.

The option “*pruning*” in Xpert Rule Analyser presents an opportunity to reduce, for example, the effects of noise in the induced knowledge tree, an option which is particularly useful when an induced tree becomes too extensive. For discrete outcome values, a statistical pruning based on Chi-square test of independence and the branches in the tree will be pruned (*cut*). The pruned knowledge tree can then be verified and the results can be compared with the non-pruned tree. An advantage is that a pruned knowledge tree will be much more feasible to interpret.

## Results

### Macro Photos

The normal sound primary teeth appeared with a whitish colour while the crowns of the DI-teeth had a slight brownish colour. Some of the DI-teeth were broken by dryness sagittally in halves as and the exposed dentine appeared compact without any normal dentinal structure showing no alignment of dentinal tubules (Figure 2a-b). The colour of the exposed dentine was yellowish to brownish shade appearing to be darker compared with the dentine in the normal primary teeth. Normal physiological resorption of the roots before exfoliation was seen in the normal primary teeth in contrast to what was seen the DI-teeth. In the extracted DI-teeth the coronal part was abraded and the pulp chambers in the roots were completely obliterated (Figure 2a-b).



**Figure 2a-b:** Un-decalcified specimens of primary teeth diagnosed Dentinogenesis Imperfecta type II seen in incident light. a) Primary incisor with obliterated pulp chamber. b) Root of primary mandibular molar (E=enamel; D=dentine; RC=root cementum; White bar=2 mm)

### XRMA Results

The full set of data from the XRMA measurements in the dentine of normal primary teeth and in DI-dentine are given in Appendix 1.

**Appendix 1:** Compiled data for carbon (C), oxygen (O), sodium (Na), magnesium (Mg), phosphorous (P), potassium (K) and calcium (Ca) in weight% from XRMA measurements in 5 primary teeth with normal dentine (N) and dentine in 7 primary teeth from patients diagnosed with Dentinogenesis Imperfecta type II (DI) for each sample (Normal=N-01 – N-05; DI=DI-01 – DI-07) in ten points (Point 1-10) from the enamel-dentine junction (Point 1) toward the pulpal dentine (Point 10). (Mean=mean value; SD=standard deviation.)

Line	A	B	C	D	Mean	SD
<b>N-01 Carbon Point</b>						
1	30.79	34.38	31.01	32.48	32.17	1.435
2	30.42	29.75	31.57	32.60	31.08	1.091
3	30.27	29.01	31.69	33.50	31.12	1.669
4	28.28	29.59	31.78	33.06	30.68	1.859
5	27.94	28.10	30.08	31.12	29.31	1.344
6	28.07	31.66	30.60	28.19	29.63	1.547
7	28.61	31.36	29.84	32.53	30.58	1.485
8	30.86	31.36	31.98	28.75	30.74	1.217
9	30.74	28.95	33.73	35.80	32.31	2.641
10	30.59	27.35	36.71	34.32	32.24	3.571
<b>N-01 Oxygen Point</b>						
1	30.43	32.52	32.55	33.42	32.23	1.100
2	34.15	29.11	28.92	29.83	30.50	2.131
3	32.51	28.39	26.96	28.74	29.15	2.053
4	30.15	27.71	27.20	27.14	28.05	1.233
5	30.56	31.95	29.32	29.16	30.25	1.122
6	30.72	26.17	27.94	29.69	28.63	1.733
7	27.82	29.94	32.41	28.38	29.64	1.777
8	31.05	29.49	28.83	26.53	28.98	1.625
9	26.12	29.34	25.49	20.46	25.35	3.183
10	29.92	28.94	27.82	28.14	28.71	0.811
<b>N-01 Sodium Point</b>						
1	0.32	0.28	0.35	0.21	0.29	0.052
2	0.26	0.42	0.35	0.28	0.33	0.063
3	0.29	0.43	0.40	0.30	0.36	0.060
4	0.38	0.39	0.42	0.37	0.39	0.019
5	0.52	0.40	0.44	0.40	0.44	0.048
6	0.45	0.35	0.37	0.32	0.37	0.046
7	0.44	0.37	0.35	0.33	0.37	0.039
8	0.33	0.38	0.31	0.25	0.32	0.046
9	0.35	0.38	0.23	0.20	0.29	0.079
10	0.51	0.37	0.20	0.27	0.34	0.117
<b>N-01 Magnesium Point</b>						
1	0.52	0.55	0.52	0.47	0.52	0.028
2	0.45	0.58	0.55	0.57	0.53	0.053
3	0.56	0.64	0.57	0.53	0.58	0.041
4	0.52	0.62	0.59	0.61	0.58	0.041
5	0.62	0.63	0.70	0.66	0.65	0.030

6	0.59	0.68	0.69	0.67	0.66	0.040
7	0.62	0.75	0.75	0.75	0.72	0.058
8	0.56	0.71	0.72	0.66	0.66	0.066
9	0.53	0.67	0.61	0.59	0.60	0.051
10	0.60	0.71	0.38	0.54	0.56	0.120
<b>N-01 Phosphorous Point</b>						
1	13.00	11.20	12.35	11.58	12.03	0.694
2	12.08	13.73	13.32	12.68	12.95	0.628
3	12.71	14.17	13.80	12.62	13.32	0.675
4	14.06	14.20	13.68	13.25	13.80	0.368
5	14.09	13.43	13.64	13.32	13.62	0.296
6	13.91	13.98	13.79	13.47	13.79	0.195
7	14.85	13.07	12.58	13.07	13.39	0.868
8	13.32	13.08	13.12	14.34	13.46	0.512
9	14.67	13.73	13.43	14.04	13.97	0.460
10	13.47	14.30	11.74	12.43	12.99	0.979
<b>N-01 Chlorine Point</b>						
1	0.09	0.13	0.13	0.18	0.13	0.033
2	0.08	0.08	0.07	0.08	0.08	0.005
3	0.06	0.07	0.07	0.07	0.07	0.006
4	0.05	0.08	0.07	0.07	0.07	0.009
5	0.05	0.05	0.07	0.06	0.06	0.006
6	0.05	0.07	0.08	0.05	0.06	0.013
7	0.07	0.06	0.06	0.06	0.06	0.003
8	0.05	0.06	0.06	0.06	0.06	0.005
9	0.07	0.09	0.05	0.06	0.07	0.015
10	0.07	0.06	0.03	0.03	0.05	0.017
<b>N-01 Potassium Point</b>						
1	0.01	0.02	0.00	0.02	0.01	0.006
2	0.01	0.00	0.01	0.01	0.01	0.003
3	0.02	0.00	0.01	0.01	0.01	0.006
4	0.01	0.01	0.01	0.01	0.01	0.003
5	0.01	0.01	0.02	0.01	0.01	0.004
6	0.02	0.00	0.02	0.02	0.02	0.007
7	0.02	0.01	0.01	0.02	0.02	0.004
8	0.01	0.02	0.01	0.01	0.01	0.004
9	0.02	0.02	0.02	0.02	0.02	0.003
10	0.02	0.02	0.01	0.01	0.02	0.004
<b>N-01 Calcium Point</b>						
1	24.84	20.92	23.09	21.63	22.62	1.500
2	22.56	26.33	25.21	23.95	24.51	1.404
3	23.59	27.29	26.50	24.24	25.40	1.535
4	26.55	27.40	26.25	25.50	26.42	0.681
5	26.21	25.42	25.74	25.28	25.66	0.358
6	26.19	27.09	26.52	27.58	26.84	0.532
7	27.57	24.44	24.00	24.85	25.22	1.393
8	23.81	24.90	24.97	29.40	25.77	2.146

9	27.49	26.80	26.44	28.84	27.39	0.916
10	24.81	28.25	23.10	24.25	25.10	1.916

Line	A	B	Mean	SD
<b>N-02 Carbon</b>				
Point	34.84	35.19	35.01	0.171
2	29.80	33.12	31.46	1.661
3	30.65	32.15	31.40	0.750
4	31.71	31.00	31.36	0.357
5	30.93	29.68	30.30	0.626
6	29.86	31.26	30.56	0.700
7	31.28	31.93	31.60	0.327
8	31.91	30.90	31.41	0.503
9	31.07	32.26	31.66	0.594
10	39.52	31.87	35.70	3.824
<b>N-02 Oxygen Point</b>				
1	31.69	32.27	31.98	0.292
2	31.98	28.34	30.16	1.820
3	28.60	28.03	28.32	0.285
4	27.64	29.43	28.54	0.894
5	29.42	26.51	27.96	1.456
6	29.48	30.62	30.05	0.569
7	28.49	22.25	25.37	3.120
8	26.32	26.24	26.28	0.041
9	29.63	29.12	29.38	0.256
10	31.91	30.44	31.18	0.734
<b>N-02 Sodium Point</b>				
1	0.26	0.26	0.26	0.001
2	0.30	0.31	0.31	0.008
3	0.35	0.28	0.32	0.032
4	0.36	0.33	0.34	0.017
5	0.26	0.34	0.30	0.039
6	0.25	0.20	0.22	0.026
7	0.25	0.25	0.25	0.001
8	0.25	0.28	0.27	0.015
9	0.22	0.17	0.20	0.025
10	0.21	0.26	0.24	0.028
<b>N-02 Magnesium Point</b>				
1	0.60	0.64	0.62	0.017
2	0.58	0.65	0.61	0.031
3	0.67	0.64	0.66	0.014
4	0.71	0.73	0.72	0.012
5	0.74	0.66	0.70	0.038
6	0.77	0.70	0.74	0.035
7	0.78	0.73	0.76	0.024
8	0.78	0.79	0.79	0.004
9	0.75	0.71	0.73	0.024

10	0.57	0.74	0.66	0.086
<b>N-02 Phosphorous Point</b>				
1	11.53	11.24	11.39	0.148
2	13.23	13.00	13.12	0.115
3	13.75	13.46	13.60	0.144
4	13.72	13.48	13.60	0.117
5	13.55	14.55	14.05	0.504
6	13.86	13.27	13.57	0.298
7	13.65	14.97	14.31	0.660
8	14.01	14.39	14.20	0.189
9	13.41	13.02	13.22	0.195
10	9.83	12.71	11.27	1.443
<b>N-02 Chlorine Point</b>				
1	0.07	0.07	0.07	0.001
2	0.04	0.04	0.04	0.002
3	0.05	0.05	0.05	0.001
4	0.03	0.04	0.04	0.007
5	0.03	0.04	0.04	0.005
6	0.05	0.04	0.04	0.009
7	0.03	0.03	0.03	0.001
8	0.04	0.03	0.04	0.007
9	0.05	0.03	0.04	0.009
10	0.08	0.04	0.06	0.017
<b>N-02 Potassium Point</b>				
1	0.00	0.00	0.00	0.000
2	0.01	0.00	0.01	0.001
3	0.01	0.01	0.01	0.002
4	0.01	0.01	0.01	0.001
5	0.01	0.01	0.01	0.003
6	0.00	0.00	0.00	0.000
7	0.01	0.02	0.01	0.005
8	0.01	0.01	0.01	0.000
9	0.02	0.01	0.02	0.016
10	0.00	0.00	0.00	0.000
<b>N-02 Calcium Point</b>				
1	21.00	20.34	20.67	0.331
2	24.06	24.53	24.30	0.236
3	25.93	25.37	25.65	0.277
4	25.82	24.98	25.40	0.422
5	25.07	28.22	26.64	1.575
6	25.71	23.91	24.81	0.901
7	25.52	29.82	27.67	2.153
8	26.66	27.35	27.00	0.342
9	24.84	24.68	24.76	0.079
10	17.88	23.92	20.90	3.019

Line	A	B	C	D	Mean	SD
<b>N-03 Carbon Point</b>						
1	35.37	35.08	35.29	35.23	35.24	0.108
2	27.34	28.17	32.67	33.76	30.49	2.772
3	27.18	27.83	33.82	34.52	30.84	3.352
4	26.58	27.76	27.90	30.84	28.27	1.570
5	28.03	27.69	29.22	33.92	29.72	2.494
6	27.05	29.13	30.46	34.08	30.18	2.559
7	29.50	28.31	29.45	33.78	30.26	2.090
8	27.52	30.16	30.73	29.37	29.44	1.212
9	26.60	29.62	30.48	32.30	29.75	2.058
10	26.06	28.42	29.38	32.07	28.98	2.154
<b>N-03 Oxygen Point</b>						
1	26.75	26.23	25.49	23.75	25.55	1.134
2	29.91	28.98	23.26	25.88	27.01	2.631
3	30.00	30.46	26.95	25.02	28.11	2.233
4	31.57	29.86	26.92	27.15	28.88	1.938
5	30.87	29.03	28.20	24.81	28.22	2.196
6	31.43	26.82	26.91	25.60	27.69	2.218
7	29.52	27.92	27.76	24.00	27.30	2.027
8	30.90	27.05	26.60	27.37	27.98	1.710
9	31.02	28.53	25.76	23.78	27.27	2.743
10	32.30	28.27	26.16	19.40	26.53	4.673
<b>N-03 Sodium Point</b>						
1	0.26	0.29	0.20	0.25	0.25	0.030
2	0.28	0.37	0.28	0.24	0.29	0.049
3	0.33	0.31	0.22	0.27	0.28	0.041
4	0.33	0.28	0.31	0.29	0.30	0.018
5	0.30	0.29	0.33	0.29	0.30	0.017
6	0.27	0.29	0.28	0.27	0.28	0.011
7	0.27	0.27	0.27	0.25	0.26	0.008
8	0.27	0.29	0.24	0.25	0.26	0.022
9	0.25	0.26	0.26	0.17	0.23	0.038
10	0.23	0.22	0.20	0.10	0.19	0.053
<b>N-03 Magnesium Point</b>						
1	0.51	0.58	0.46	0.54	0.52	0.043
2	0.58	0.60	0.61	0.59	0.60	0.010
3	0.68	0.64	0.58	0.62	0.63	0.036
4	0.72	0.65	0.62	0.67	0.66	0.036
5	0.71	0.75	0.70	0.61	0.69	0.052
6	0.81	0.77	0.66	0.57	0.70	0.091
7	0.81	0.87	0.74	0.66	0.77	0.076
8	0.82	0.86	0.71	0.73	0.78	0.063
9	0.87	0.79	0.79	0.68	0.78	0.059
10	0.84	0.83	0.87	0.55	0.77	0.131
<b>N-03 Phosphorous Point</b>						

1	12.46	12.70	12.83	13.46	12.86	0.369
2	14.25	14.21	14.46	13.47	14.10	0.376
3	14.18	14.05	13.05	13.44	13.68	0.456
4	13.79	14.22	14.71	13.92	14.16	0.351
5	13.43	14.47	14.15	13.67	13.93	0.407
6	13.86	14.65	13.82	13.41	13.94	0.449
7	13.68	14.53	14.25	13.95	14.10	0.320
8	13.85	14.30	13.90	14.32	14.09	0.220
9	14.23	13.65	14.50	14.29	14.17	0.319
10	14.01	14.39	14.74	14.86	14.50	0.331
<b>N-03 Chlorine Point</b>						
1	0.03	0.04	0.05	0.04	0.04	0.010
2	0.04	0.03	0.03	0.03	0.03	0.003
3	0.04	0.04	0.04	0.03	0.04	0.005
4	0.03	0.05	0.03	0.04	0.04	0.007
5	0.03	0.03	0.04	0.03	0.03	0.005
6	0.02	0.04	0.03	0.03	0.03	0.006
7	0.03	0.03	0.04	0.04	0.04	0.005
8	0.03	0.03	0.05	0.03	0.04	0.010
9	0.02	0.03	0.03	0.05	0.03	0.009
10	0.04	0.04	0.03	0.07	0.05	0.016
<b>N-03 Potassium Point</b>						
1	0.00	0.01	0.00	0.00	0.00	0.003
2	0.00	0.01	0.00	0.01	0.01	0.003
3	0.00	0.00	0.00	0.00	0.00	0.001
4	0.01	0.01	0.00	0.01	0.01	0.003
5	0.00	0.00	0.01	0.00	0.00	0.003
6	0.01	0.01	0.00	0.01	0.01	0.004
7	0.00	0.01	0.02	0.00	0.01	0.007
8	0.02	0.00	0.01	0.01	0.01	0.006
9	0.00	0.00	0.01	0.01	0.01	0.004
10	0.00	0.00	0.01	0.01	0.01	0.005
<b>N-03 Calcium Point</b>						
1	24.64	25.08	25.67	26.73	25.53	0.785
2	27.59	27.63	28.69	26.03	27.48	0.949
3	27.60	26.68	25.33	26.09	26.43	0.832
4	26.97	27.18	29.51	27.09	27.69	1.056
5	26.63	27.74	27.35	26.67	27.10	0.468
6	26.55	28.29	27.86	26.02	27.18	0.927
7	26.20	28.06	27.48	27.32	27.27	0.676
8	26.59	27.32	27.76	27.91	27.40	0.513
9	27.00	27.14	28.18	28.73	27.76	0.722
10	26.53	27.84	28.61	32.94	28.98	2.407

Line	A	B	Mean	SD
<b>N-04 Carbon Point</b>				
1	34.92	34.25	34.59	0.332
2	31.99	32.21	32.10	0.108
3	29.30	29.44	29.37	0.070
4	32.75	28.20	30.47	2.275
5	32.04	26.53	29.28	2.757
6	31.31	30.88	31.09	0.215
7	33.26	31.71	32.48	0.779
8	33.88	31.11	32.50	1.381
9	34.55	35.05	34.80	0.250
10	32.14	31.89	32.01	0.128
<b>N-04 Oxygen Point</b>				
1	32.38	31.70	32.04	0.335
2	31.54	29.90	30.72	0.818
3	27.72	26.41	27.07	0.657
4	27.44	24.93	26.18	1.258
5	29.18	30.22	29.70	0.519
6	27.67	25.19	26.43	1.239
7	25.97	24.86	25.42	0.555
8	25.72	24.11	24.91	0.804
9	24.85	27.24	26.05	1.195
10	31.79	23.43	27.61	4.180
<b>N-04 Sodium Point</b>				
1	0.22	0.23	0.23	0.005
2	0.20	0.22	0.21	0.013
3	0.26	0.24	0.25	0.013
4	0.26	0.24	0.25	0.009
5	0.26	0.24	0.25	0.007
6	0.25	0.23	0.24	0.014
7	0.25	0.21	0.23	0.019
8	0.24	0.25	0.25	0.009
9	0.23	0.22	0.23	0.007
10	0.22	0.18	0.20	0.021
<b>N-04 Magnesium Point</b>				
1	0.53	0.54	0.54	0.006
2	0.49	0.53	0.51	0.020
3	0.56	0.57	0.56	0.009
4	0.62	0.66	0.64	0.020
5	0.68	0.63	0.65	0.023
6	0.73	0.68	0.70	0.025
7	0.77	0.70	0.73	0.037
8	0.76	0.84	0.80	0.039
9	0.78	0.77	0.77	0.005
10	0.85	0.76	0.80	0.045
<b>N-04 Phosphorous Point</b>				
1	11.29	11.67	11.48	0.193

2	12.57	12.84	12.70	0.132
3	14.32	14.53	14.42	0.110
4	13.50	14.94	14.22	0.719
5	13.14	14.23	13.69	0.546
6	13.80	14.36	14.08	0.281
7	13.63	14.14	13.89	0.253
8	13.48	14.97	14.22	0.746
9	13.66	12.64	13.15	0.508
10	12.45	14.65	13.55	1.101
<b>N-04 Chlorine Point</b>				
1	0.03	0.04	0.04	0.005
2	0.04	0.02	0.03	0.009
3	0.04	0.03	0.04	0.004
4	0.03	0.04	0.03	0.005
5	0.04	0.04	0.04	0.001
6	0.04	0.03	0.03	0.003
7	0.04	0.04	0.04	0.000
8	0.05	0.04	0.05	0.005
9	0.05	0.05	0.05	0.001
10	0.03	0.02	0.03	0.003
<b>N-04 Potassium Point</b>				
1	0.00	0.00	0.00	0.002
2	0.01	0.01	0.01	0.003
3	0.01	0.00	0.01	0.006
4	0.00	0.00	0.00	0.001
5	0.01	0.00	0.00	0.003
6	0.00	0.00	0.00	0.002
7	0.01	0.00	0.01	0.004
8	0.02	0.01	0.01	0.004
9	0.01	0.01	0.01	0.001
10	0.01	0.00	0.00	0.001
<b>N-04 Calcium Point</b>				
1	20.63	21.55	21.09	0.463
2	23.16	24.26	23.71	0.551
3	27.79	28.77	28.28	0.491
4	25.40	31.00	28.20	2.798
5	24.65	28.10	26.38	1.724
6	26.20	28.64	27.42	1.218
7	26.06	28.34	27.20	1.142
8	25.86	28.66	27.26	1.399
9	25.87	24.02	24.94	0.923
10	22.51	29.07	25.79	3.278

Line	A	B	C	D	Mean	SD
<b>N-05 Carbon Point</b>						
1	31.56	32.46	29.53	29.23	30.70	1.356
2	29.46	30.66	28.17	29.14	29.36	0.890
3	30.86	30.50	26.56	29.19	29.28	1.691
4	29.96	30.25	28.74	29.22	29.54	0.598
5	27.79	30.26	27.66	29.47	28.80	1.104
6	30.57	29.81	27.59	30.93	29.72	1.298
7	27.82	25.73	26.32	29.25	27.28	1.368
8	28.59	28.47	29.88	31.01	29.49	1.037
9	28.00	30.23	27.92	32.29	29.61	1.805
10	30.20	34.53	39.35	32.74	34.21	3.347
<b>N-05 Oxygen Point</b>						
1	24.98	29.61	23.14	25.88	25.90	2.356
2	30.65	26.70	27.82	25.18	27.59	2.003
3	28.67	29.78	28.86	28.11	28.86	0.602
4	26.02	27.60	30.28	26.52	27.60	1.646
5	22.53	30.11	30.36	27.29	27.57	3.150
6	27.93	31.28	29.44	25.97	28.65	1.951
7	25.00	34.12	27.58	24.56	27.82	3.815
8	25.38	31.29	23.33	26.20	26.55	2.931
9	29.99	27.14	24.55	23.51	26.30	2.507
10	27.19	22.67	24.29	26.48	25.16	1.792
<b>N-05 Sodium Point</b>						
1	0.22	0.21	0.19	0.28	0.23	0.035
2	0.25	0.34	0.26	0.29	0.28	0.035
3	0.20	0.26	0.25	0.24	0.24	0.022
4	0.18	0.35	0.25	0.25	0.26	0.058
5	0.32	0.24	0.23	0.24	0.26	0.036
6	0.21	0.26	0.30	0.23	0.25	0.036
7	0.24	0.26	0.25	0.22	0.24	0.016
8	0.28	0.22	0.19	0.23	0.23	0.032
9	0.31	0.21	0.14	0.13	0.20	0.073
10	0.38	0.11	0.14	0.20	0.21	0.106
<b>N-05 Magnesium Point</b>						
1	0.60	0.56	0.43	0.87	0.62	0.161
2	0.53	0.63	0.63	0.64	0.61	0.048
3	0.56	0.64	0.63	0.66	0.62	0.038
4	0.60	0.71	0.72	0.67	0.67	0.047
5	0.70	0.73	0.68	0.65	0.69	0.027
6	0.72	0.74	0.79	0.68	0.73	0.038
7	0.78	0.75	0.75	0.69	0.74	0.030
8	0.80	0.78	0.75	0.86	0.80	0.041
9	0.90	0.74	0.82	0.84	0.82	0.059
10	0.69	0.55	0.52	0.63	0.60	0.066
<b>N-05 Phosphorous Point</b>						
1	14.67	12.81	14.83	14.33	14.16	0.801

2	13.70	14.18	14.36	14.74	14.24	0.376
3	14.09	13.57	15.04	14.36	14.26	0.529
4	15.37	14.21	13.81	14.59	14.50	0.577
5	16.28	13.48	14.17	14.31	14.56	1.039
6	14.30	13.23	14.35	14.35	14.06	0.476
7	15.45	13.33	14.92	14.70	14.60	0.781
8	15.28	13.76	15.02	14.13	14.55	0.623
9	14.25	14.43	15.67	14.45	14.70	0.563
10	14.29	13.18	12.06	13.30	13.21	0.792
<b>N-05 Chlorine Point</b>						
1	0.08	0.09	0.19	0.05	0.10	0.051
2	0.04	0.05	0.06	0.04	0.05	0.008
3	0.04	0.04	0.04	0.04	0.04	0.004
4	0.04	0.05	0.04	0.03	0.04	0.009
5	0.08	0.05	0.04	0.03	0.05	0.017
6	0.02	0.05	0.04	0.03	0.04	0.011
7	0.04	0.05	0.05	0.04	0.04	0.003
8	0.03	0.05	0.04	0.04	0.04	0.005
9	0.06	0.04	0.10	0.04	0.06	0.024
10	0.08	0.27	0.24	0.03	0.15	0.099
<b>N-05 Potassium Point</b>						
1	0.01	0.00	0.01	0.02	0.01	0.006
2	0.01	0.01	0.00	0.02	0.01	0.007
3	0.02	0.01	0.02	0.01	0.01	0.004
4	0.03	0.00	0.01	0.02	0.01	0.012
5	0.03	0.00	0.01	0.02	0.02	0.011
6	0.01	0.00	0.01	0.00	0.01	0.004
7	0.02	0.01	0.01	0.03	0.02	0.006
8	0.02	0.01	0.01	0.01	0.01	0.002
9	0.02	0.01	0.01	0.02	0.01	0.004
10	-0.01	0.01	0.01	0.03	0.01	0.015
<b>N-05 Calcium Point</b>						
1	27.87	24.25	31.67	29.33	28.28	2.694
2	25.36	27.43	28.70	29.95	27.86	1.694
3	25.56	25.20	28.61	27.39	26.69	1.385
4	27.79	26.84	26.16	28.71	27.37	0.964
5	32.28	25.13	26.85	27.99	28.06	2.640
6	26.25	24.62	27.49	27.80	26.54	1.250
7	30.65	25.76	30.12	30.51	29.26	2.029
8	29.62	25.42	30.78	27.52	28.33	2.048
9	26.47	27.20	30.79	28.71	28.29	1.652
10	27.18	28.68	23.40	26.59	26.46	1.924
<b>DI-01 Carbon Point</b>						
1	28.44	27.11	28.07	27.20	27.70	0.567
2	28.95	26.66	26.27	27.07	27.24	1.029
3	29.20	28.48	30.97	28.03	29.17	1.121
4	27.73	28.79	29.26	28.25	28.51	0.576



5	27.76	29.53	26.47	29.75	28.38	1.344
6	28.71	29.45	29.21	28.45	28.95	0.394
7	29.75	29.35	31.35	30.11	30.14	0.748
8	31.70	30.56	28.61	27.56	29.61	1.616
9	30.45	30.67	27.20	31.47	29.95	1.631
10	33.35	26.89	27.00	29.38	29.15	2.619
<b>DI-01 Oxygen Point</b>						
1	30.80	31.39	28.43	28.91	29.88	1.242
2	32.73	30.01	31.70	27.78	30.55	1.874
3	28.13	27.17	29.27	27.46	28.01	0.806
4	27.65	25.75	30.75	30.81	28.74	2.146
5	27.48	30.58	27.96	30.10	29.03	1.335
6	28.36	27.58	28.73	25.21	27.47	1.367
7	23.72	30.97	30.33	31.08	29.03	3.079
8	24.18	30.87	28.55	25.76	27.34	2.569
9	22.74	25.12	26.84	26.11	25.20	1.547
10	24.93	28.60	28.38	23.59	26.37	2.167
<b>DI-01 Sodium Point</b>						
1	0.31	0.44	0.46	0.36	0.39	0.058
2	0.26	0.35	0.32	0.40	0.33	0.051
3	0.34	0.45	0.33	0.44	0.39	0.057
4	0.30	0.51	0.28	0.25	0.33	0.103
5	0.34	0.34	0.44	0.29	0.35	0.053
6	0.32	0.39	0.35	0.41	0.37	0.036
7	0.30	0.33	0.28	0.29	0.30	0.018
8	0.29	0.29	0.34	0.41	0.33	0.050
9	0.28	0.39	0.43	0.37	0.37	0.052
10	0.38	0.40	0.50	0.53	0.45	0.063
<b>DI-01 Magnesium Point</b>						
1	0.71	0.71	0.76	0.68	0.72	0.029
2	0.63	0.70	0.65	0.66	0.66	0.023
3	0.57	0.51	0.55	0.64	0.57	0.047
4	0.59	0.55	0.63	0.64	0.60	0.036
5	0.57	0.41	0.54	0.66	0.55	0.089
6	0.58	0.53	0.55	0.73	0.60	0.080
7	0.29	0.58	0.60	0.55	0.51	0.126
8	0.25	0.58	0.60	0.54	0.49	0.139
9	0.24	0.60	0.57	0.45	0.47	0.143
10	0.24	0.68	0.59	0.46	0.49	0.163
<b>DI-01 Phosphorous Point</b>						
1	13.63	13.66	14.25	14.59	14.03	0.407
2	13.00	14.59	14.15	15.06	14.20	0.761
3	14.42	14.78	13.28	14.58	14.26	0.581
4	14.81	15.21	13.60	13.78	14.35	0.678
5	15.02	13.64	14.97	13.61	14.31	0.684
6	14.62	14.46	14.09	15.26	14.61	0.421
7	14.81	13.56	12.85	13.09	13.57	0.755

8	14.25	13.03	14.33	15.26	14.22	0.793
9	14.97	14.58	14.99	13.89	14.61	0.443
10	13.56	14.72	14.63	15.25	14.54	0.614
<b>DI-01 Chlorine Point</b>						
1	0.13	0.08	0.10	0.06	0.09	0.028
2	0.06	0.04	0.04	0.04	0.05	0.007
3	0.04	0.04	0.04	0.04	0.04	0.003
4	0.05	0.05	0.05	0.04	0.04	0.005
5	0.05	0.05	0.06	0.04	0.05	0.004
6	0.05	0.06	0.05	0.04	0.05	0.009
7	0.05	0.04	0.03	0.04	0.04	0.008
8	0.08	0.05	0.05	0.06	0.06	0.012
9	0.14	0.06	0.03	0.05	0.07	0.039
10	0.10	0.06	0.06	0.05	0.07	0.021
<b>DI-01 Potassium Point</b>						
1	0.03	0.01	0.03	0.01	0.02	0.008
2	0.00	0.00	0.00	0.01	0.00	0.003
3	0.00	0.01	0.00	0.00	0.00	0.004
4	0.01	0.01	0.01	0.00	0.01	0.004
5	0.00	0.01	0.01	0.00	0.01	0.006
6	0.01	0.01	0.01	0.01	0.01	0.003
7	0.01	0.00	0.02	0.01	0.01	0.005
8	0.00	0.01	0.00	0.01	0.00	0.003
9	0.01	0.01	0.00	0.00	0.01	0.006
10	0.01	0.00	0.00	0.01	0.00	0.003
<b>DI-01 Calcium Point</b>						
1	25.95	26.60	27.90	28.18	27.70	0.567
2	24.37	27.68	26.86	28.99	27.24	1.029
3	27.30	28.57	25.57	28.80	29.17	1.121
4	28.87	29.12	25.42	26.25	28.51	0.576
5	28.78	25.43	29.56	25.53	28.38	1.344
6	27.37	27.53	27.00	29.89	28.95	0.394
7	31.07	25.17	24.54	24.84	30.14	0.748
8	29.26	24.63	27.53	30.41	29.61	1.616
9	31.16	28.57	29.95	27.67	29.95	1.631
10	27.43	28.66	28.84	30.74	29.15	2.619

Line	A	B	C	D	E	F	Mean	SD
<b>DI-02 Carbon Point</b>								
1	29.49	33.78	38.13	28.25	28.73	29.32	31.28	3.556
2	27.28	29.73	35.72	26.80	27.21	29.90	29.44	3.064
3	27.44	30.92	31.80	26.69	27.89	27.79	28.76	1.900
4	29.60	28.95	32.25	26.72	27.77	28.03	28.89	1.756
5	32.05	27.78	30.44	26.88	27.78	27.40	28.72	1.869
6	30.63	30.44	32.33	27.07	27.92	28.47	29.48	1.809
7	30.64	30.05	30.65	27.75	28.89	29.30	29.55	1.033
8	30.12	30.49	29.58	33.29	28.05	28.83	30.06	1.653
9	32.24	29.60	29.87	30.58	31.32	28.26	30.31	1.272
10	29.73	30.68	30.59	30.53	30.53	29.64	30.28	0.425
<b>DI-02 Oxygen Point</b>								
1	33.33	31.56	29.02	30.71	30.17	32.58	31.23	1.450
2	31.51	34.09	32.07	32.43	30.22	27.04	31.23	2.199
3	33.15	34.63	29.44	30.59	30.24	28.23	31.05	2.185
4	31.33	32.24	32.07	30.77	37.10	30.28	32.30	2.252
5	32.39	31.27	30.60	29.77	29.50	29.20	30.45	1.111
6	34.01	33.69	31.83	28.26	26.53	29.50	30.64	2.767
7	34.88	29.79	35.18	28.26	29.53	28.84	31.08	2.837
8	32.49	34.08	29.07	27.68	31.76	30.58	30.94	2.130
9	33.52	30.45	33.10	28.62	28.18	30.99	30.81	2.020
10	30.97	28.94	28.79	30.23	28.68	29.72	29.55	0.839
<b>DI-02 Sodium Point</b>								
1	0.46	0.34	0.31	0.40	0.45	0.39	0.39	0.053
2	0.45	0.33	0.28	0.35	0.42	0.40	0.37	0.057
3	0.36	0.30	0.32	0.34	0.37	0.41	0.35	0.036
4	0.35	0.35	0.29	0.34	0.30	0.35	0.33	0.026
5	0.30	0.36	0.32	0.31	0.38	0.38	0.34	0.035
6	0.30	0.30	0.27	0.30	0.41	0.39	0.33	0.053
7	0.27	0.27	0.27	0.54	0.45	0.45	0.37	0.110
8	0.33	0.28	0.43	0.48	0.40	0.33	0.37	0.068
9	0.36	0.52	0.33	0.54	0.50	0.36	0.44	0.086
10	0.53	0.57	0.54	0.51	0.55	0.47	0.53	0.032
<b>DI-02 Magnesium Point</b>								
1	0.75	0.30	0.29	0.69	0.49	0.48	0.50	0.174
2	0.59	0.71	0.54	0.69	0.59	0.49	0.60	0.076
3	0.47	0.69	0.60	0.69	0.54	0.51	0.58	0.084
4	0.37	0.68	0.63	0.63	0.50	0.46	0.55	0.112
5	0.37	0.66	0.61	0.60	0.47	0.46	0.53	0.101
6	0.54	0.65	0.60	0.53	0.45	0.39	0.53	0.086
7	0.52	0.54	0.58	0.56	0.45	0.39	0.51	0.066
8	0.61	0.56	0.60	0.48	0.47	0.38	0.52	0.080
9	0.58	0.61	0.49	0.50	0.50	0.38	0.51	0.073
10	0.65	0.59	0.56	0.48	0.51	0.39	0.53	0.082
<b>DI-02 Phosphorous Point</b>								

1	12.16	11.12	10.50	13.38	13.14	12.25	12.09	1.022
2	13.78	12.17	10.72	13.50	13.86	13.66	12.95	1.147
3	13.46	11.76	12.75	13.96	13.58	14.20	13.28	0.819
4	13.01	13.13	11.98	13.87	11.83	13.49	12.89	0.746
5	11.86	13.16	12.85	14.06	13.68	13.99	13.27	0.762
6	12.12	12.21	12.12	14.19	14.28	13.39	13.05	0.945
7	12.28	13.93	11.94	14.07	13.22	13.43	13.14	0.792
8	12.68	11.98	13.51	12.64	13.05	13.18	12.84	0.487
9	11.39	12.92	12.61	13.27	12.99	13.14	12.72	0.630
10	12.81	13.10	13.25	12.78	13.20	13.12	13.04	0.183
<b>DI-02 Chlorine Point</b>								
1	0.06	0.05	0.03	0.03	0.03	0.03	0.04	0.012
2	0.11	0.04	0.08	0.04	0.05	0.04	0.06	0.027
3	0.05	0.04	0.06	0.05	0.03	0.05	0.05	0.008
4	0.06	0.04	0.04	0.05	0.04	0.04	0.05	0.007
5	0.05	0.05	0.06	0.04	0.04	0.04	0.05	0.006
6	0.04	0.04	0.04	0.06	0.04	0.05	0.05	0.008
7	0.07	0.04	0.05	0.04	0.04	0.05	0.05	0.009
8	0.06	0.04	0.05	0.04	0.06	0.04	0.05	0.009
9	0.04	0.05	0.05	0.03	0.02	0.05	0.04	0.011
10	0.04	0.04	0.06	0.05	0.02	0.05	0.04	0.011
<b>DI-02 Potassium Point</b>								
1	0.02	0.02	0.01	0.00	0.01	0.01	0.01	0.005
2	0.02	0.00	0.01	0.00	0.01	0.01	0.01	0.006
3	0.03	0.00	0.00	0.00	0.01	0.01	0.01	0.009
4	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.003
5	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.004
6	0.01	0.02	0.00	0.00	0.02	0.01	0.01	0.008
7	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.002
8	0.01	0.01	0.01	0.01	0.00	0.02	0.01	0.005
9	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.006
10	0.01	0.00	0.02	0.00	0.00	0.00	0.01	0.006
<b>DI-02 Calcium Point</b>								
1	23.73	22.83	21.70	26.53	26.97	24.93	24.45	1.899
2	26.26	22.93	20.57	26.19	27.64	28.46	25.34	2.744
3	25.03	21.66	25.04	27.69	27.34	28.80	25.93	2.349
4	25.27	24.62	22.73	27.60	22.45	27.34	25.00	2.006
5	22.97	26.72	25.13	28.35	28.14	28.52	26.64	2.021
6	22.35	22.64	22.81	29.58	30.35	27.80	25.92	3.407
7	21.36	25.38	21.31	28.77	27.41	27.54	25.29	2.92
8	23.71	22.56	26.74	25.38	26.21	26.64	25.21	1.564
9	21.88	25.84	23.55	26.43	26.49	26.81	25.17	1.822
10	25.25	26.09	26.20	25.42	26.51	26.62	26.02	0.515

Line	A	B	C	D	Mean	SD
<b>DI-03 Carbon Point (MV=missing value)</b>						
1	30.39	34.97	34.32	37.15	34.21	2.441
2	27.65	27.45	32.71	31.97	29.94	2.413
3	27.48	27.44	30.66	32.01	29.40	1.997
4	29.51	26.30	31.57	34.34	30.43	2.939
5	29.41	26.65	30.53	36.46	30.76	3.578
6	26.11	26.12	35.59	36.58	31.10	4.998
7	25.11	28.80	33.63	36.54	31.02	4.394
8	26.02	25.65	34.11	34.58	30.09	4.260
9	24.40	26.72	34.83	34.27	30.05	4.573
10	MV	32.33	37.40	35.37	35.03	2.086
<b>DI-03 Oxygen Point (MV=missing value)</b>						
1	31.94	25.07	28.54	25.54	27.77	2.749
2	27.94	28.33	23.17	24.02	25.86	2.292
3	27.63	26.14	25.38	23.83	25.74	1.371
4	25.77	28.32	27.47	27.73	27.33	0.948
5	28.36	28.55	27.32	27.59	27.95	0.512
6	29.20	29.12	26.14	27.60	28.02	1.257
7	32.02	25.51	25.25	28.36	27.78	2.734
8	28.08	27.32	20.61	29.92	26.48	3.520
9	31.52	29.22	22.82	28.97	28.13	3.225
10	MV	26.28	29.44	25.83	27.18	1.604
<b>DI-03 Sodium Point (MV=missing value)</b>						
1	0.30	0.25	0.22	0.22	0.25	0.032
2	0.25	0.27	0.17	0.18	0.22	0.043
3	0.24	0.29	0.21	0.18	0.23	0.041
4	0.26	0.23	0.25	0.15	0.22	0.043
5	0.20	0.27	0.24	0.17	0.22	0.039
6	0.23	0.22	0.18	0.21	0.21	0.018
7	0.22	0.29	0.23	0.19	0.23	0.033
8	0.21	0.24	0.22	0.16	0.21	0.030
9	0.27	0.27	0.19	0.18	0.23	0.042
10	MV	0.26	0.18	0.20	0.21	0.035
<b>DI-03 Magnesium Point (MV=missing value)</b>						
1	0.47	0.46	0.45	0.40	0.45	0.027
2	0.47	0.48	0.44	0.38	0.44	0.038
3	0.45	0.52	0.48	0.39	0.46	0.048
4	0.45	0.53	0.49	0.37	0.46	0.060
5	0.46	0.54	0.53	0.41	0.48	0.053
6	0.47	0.55	0.49	0.43	0.48	0.043
7	0.50	0.48	0.50	0.39	0.47	0.045
8	0.47	0.53	0.48	0.43	0.48	0.037
9	0.52	0.49	0.48	0.43	0.48	0.031
10	MV	0.46	0.40	0.43	0.43	0.023
<b>DI-03 Phosphorous Point (MV=missing value)</b>						
1	12.71	13.28	12.43	12.05	12.62	0.448

2	14.61	14.51	14.52	14.05	14.42	0.220
3	14.79	15.34	14.57	13.98	14.67	0.488
4	14.73	15.08	13.69	12.90	14.10	0.863
5	14.21	15.09	14.12	12.23	13.91	1.041
6	14.88	15.04	13.03	12.16	13.78	1.225
7	14.62	14.82	13.81	11.80	13.76	1.197
8	15.25	15.75	14.87	12.15	14.50	1.395
9	14.84	14.80	14.14	12.47	14.06	0.963
10	MV	13.66	11.20	13.05	12.64	1.049
<b>DI-03 Chlorine Point (MV=missing value)</b>						
1	0.04	0.05	0.05	0.05	0.05	0.003
2	0.05	0.05	0.06	0.05	0.05	0.004
3	0.05	0.06	0.06	0.06	0.06	0.007
4	0.05	0.05	0.05	0.04	0.05	0.006
5	0.06	0.05	0.06	0.04	0.05	0.009
6	0.06	0.05	0.05	0.05	0.05	0.004
7	0.06	0.06	0.04	0.04	0.05	0.008
8	0.07	0.06	0.06	0.03	0.06	0.013
9	0.08	0.05	0.05	0.02	0.05	0.020
10	MV	0.13	0.06	0.05	0.08	0.037
<b>DI-03 Potassium Point (MV=missing value)</b>						
1	0.00	0.00	0.00	0.01	0.00	0.002
2	0.00	0.01	0.00	0.00	0.00	0.006
3	0.01	0.01	0.00	0.00	0.00	0.003
4	0.00	0.00	0.01	0.00	0.00	0.003
5	0.00	0.00	0.00	0.00	0.00	0.001
6	0.00	0.00	0.01	0.00	0.00	0.005
7	0.00	0.00	0.00	0.00	0.00	0.002
8	0.01	0.00	0.00	0.01	0.00	0.004
9	0.00	0.00	0.00	0.00	0.00	0.000
10	MV	0.00	0.00	0.00	0.00	0.002
<b>DI-03 Calcium Point (MV=missing value)</b>						
1	24.16	25.92	24.00	24.59	24.67	0.754
2	29.05	28.91	28.92	29.38	29.06	0.189
3	29.36	30.19	28.64	29.56	29.44	0.554
4	29.24	29.49	26.46	24.47	27.42	2.076
5	27.29	28.86	27.21	23.10	26.61	2.134
6	29.06	28.91	24.51	22.99	26.37	2.673
7	27.47	30.05	26.55	22.67	26.68	2.650
8	29.91	30.44	29.64	22.71	28.18	3.166
9	28.38	28.45	27.50	23.67	27.00	1.958
10	MV	26.88	21.32	25.07	24.42	2.313

Line	A	B	C	D	E	Mean	SD
<b>DI-04 Carbon Point</b>							
1	34.01	31.65	31.61	31.77	35.63	32.94	1.622
2	30.29	29.38	29.48	31.59	30.50	30.25	0.800
3	28.87	27.91	29.88	26.25	28.82	28.34	1.220
4	27.27	31.30	30.20	30.61	30.27	29.93	1.387
5	27.93	28.11	29.19	30.10	31.05	29.27	1.185
6	26.93	29.44	32.51	30.18	32.63	30.34	2.116
7	29.37	29.65	30.02	32.78	33.46	31.06	1.709
8	31.71	31.04	35.00	32.49	30.99	32.25	1.479
9	30.95	31.04	36.12	35.43	32.06	33.12	2.214
10	30.85	35.02	35.26	33.66	32.06	33.37	1.698
<b>DI-04 Oxygen Point</b>							
1	29.91	31.91	30.96	30.33	29.60	30.54	0.824
2	25.32	28.94	26.09	28.70	28.93	27.59	1.565
3	26.70	28.51	25.50	31.36	31.97	28.81	2.530
4	29.02	31.46	25.31	27.57	23.20	27.31	2.865
5	29.81	28.07	27.81	26.99	23.21	27.18	2.186
6	28.79	28.79	30.52	29.11	27.43	28.93	0.985
7	30.68	29.83	28.93	26.55	29.29	29.06	1.386
8	30.28	25.82	28.09	26.45	29.82	28.09	1.766
9	29.40	27.30	25.79	28.35	27.13	27.59	1.215
10	31.67	28.50	26.09	26.13	26.16	27.71	2.183
<b>DI-04 Sodium Point</b>							
1	0.36	0.42	0.36	0.29	0.50	0.39	0.069
2	0.45	0.35	0.47	0.30	0.36	0.38	0.062
3	0.45	0.36	0.41	0.82	0.37	0.48	0.174
4	0.68	0.27	0.51	0.48	0.40	0.47	0.135
5	0.62	0.36	0.65	0.71	0.65	0.60	0.122
6	0.80	0.43	0.45	0.65	0.57	0.58	0.136
7	0.80	0.52	0.91	0.69	0.60	0.71	0.140
8	0.56	0.75	0.59	0.92	0.70	0.70	0.129
9	0.67	0.84	0.53	0.53	0.70	0.65	0.119
10	0.55	0.57	0.86	0.79	0.72	0.70	0.119
<b>DI-04 Magnesium Point</b>							
1	0.54	0.49	0.52	0.55	0.65	0.55	0.056
2	0.44	0.49	0.61	0.65	0.69	0.57	0.096
3	0.43	0.48	0.49	1.12	0.93	0.69	0.281
4	0.66	0.48	0.49	0.60	0.68	0.58	0.081
5	0.62	0.59	0.57	0.60	0.73	0.62	0.057
6	0.59	0.59	0.50	0.60	0.59	0.57	0.037
7	0.65	0.60	0.72	0.54	0.70	0.64	0.065
8	0.63	0.55	0.50	0.85	0.77	0.66	0.132
9	0.49	0.63	0.55	0.66	0.70	0.61	0.075
10	0.52	0.47	0.55	0.57	0.59	0.54	0.040
<b>DI-04 Phosphorous Point</b>							
1	12.52	12.54	13.21	13.19	12.34	12.76	0.364

2	14.76	14.59	15.22	13.73	13.87	14.43	0.559
3	14.96	14.81	15.14	15.02	14.32	14.85	0.286
4	15.42	13.19	15.19	14.47	15.56	14.77	0.875
5	15.42	14.93	14.78	15.04	15.57	15.15	0.298
6	15.53	14.85	13.50	14.87	14.74	14.70	0.660
7	14.26	15.03	14.81	14.60	14.01	14.54	0.368
8	14.04	14.93	13.58	15.13	14.69	14.47	0.577
9	14.13	15.03	13.73	13.57	14.85	14.26	0.585
10	13.69	13.11	13.83	14.47	15.11	14.04	0.689
<b>DI-04 Chlorine Point</b>							
1	0.05	0.06	0.07	0.12	0.05	0.07	0.024
2	0.07	0.06	0.06	0.08	0.06	0.07	0.007
3	0.05	0.06	0.07	0.05	0.04	0.05	0.008
4	0.05	0.06	0.04	0.05	0.05	0.05	0.008
5	0.05	0.06	0.05	0.04	0.05	0.05	0.006
6	0.04	0.05	0.05	0.05	0.05	0.05	0.006
7	0.05	0.04	0.06	0.06	0.06	0.05	0.007
8	0.04	0.04	0.05	0.04	0.04	0.04	0.004
9	0.06	0.05	0.03	0.04	0.05	0.05	0.012
10	0.06	0.05	0.04	0.04	0.05	0.05	0.008
<b>DI-04 Potassium Point</b>							
1	0.00	0.00	0.00	0.00	0.02	0.01	0.009
2	0.01	0.00	0.02	0.00	0.00	0.01	0.006
3	0.00	0.01	0.00	0.00	0.01	0.01	0.005
4	0.02	0.01	0.02	0.01	0.01	0.01	0.006
5	0.02	0.00	0.03	0.03	0.01	0.02	0.010
6	0.03	0.01	0.00	0.01	0.02	0.02	0.009
7	0.01	0.02	0.00	0.01	0.02	0.01	0.006
8	0.02	0.02	0.02	0.02	0.01	0.02	0.006
9	0.01	0.01	0.03	0.01	0.01	0.01	0.006
10	0.01	0.02	0.02	0.03	0.04	0.02	0.008
<b>DI-04 Calcium Point</b>							
1	22.60	22.93	23.27	23.77	21.20	22.75	0.867
2	28.67	26.18	28.07	24.96	25.59	26.70	1.434
3	28.53	27.87	28.51	25.36	23.53	26.76	1.991
4	26.88	23.23	28.23	26.21	29.82	26.87	2.203
5	25.54	27.88	26.92	26.49	28.74	27.12	1.106
6	27.30	25.84	22.47	24.53	23.98	24.82	1.645
7	24.17	24.31	24.54	24.77	21.87	23.93	1.049
8	22.74	26.84	22.18	24.10	22.99	23.77	1.659
9	24.29	25.10	23.23	21.41	24.49	23.70	1.294
10	22.64	22.25	23.36	24.32	25.28	23.57	1.108

Line	A	B	Mean	SD
<b>DI-05 Carbon Point</b>				
1	27.57	22.94	25.25	2.318
2	25.37	21.57	23.47	1.902
3	24.66	22.73	23.70	0.967
4	24.14	22.14	23.14	0.998
5	23.43	22.17	22.80	0.628
6	24.61	23.95	24.28	0.329
7	24.82	24.74	24.78	0.041
8	24.73	24.02	24.37	0.353
9	23.61	24.67	24.14	0.533
10	25.45	24.91	25.18	0.266
<b>DI-05 Oxygen Point</b>				
1	32.04	39.19	35.61	3.576
2	33.48	40.20	36.84	3.364
3	29.39	32.90	31.14	1.757
4	30.37	37.99	34.18	3.809
5	35.64	37.15	36.39	0.757
6	33.43	33.32	33.37	0.055
7	31.76	31.08	31.42	0.337
8	33.03	31.09	32.06	0.971
9	33.94	27.91	30.93	3.013
10	35.21	28.14	31.68	3.533
<b>DI-05 Sodium Point</b>				
1	0.48	0.41	0.44	0.031
2	0.45	0.39	0.42	0.031
3	0.43	0.50	0.47	0.038
4	0.40	0.67	0.53	0.137
5	0.43	0.40	0.41	0.015
6	0.52	0.43	0.48	0.043
7	0.47	0.61	0.54	0.068
8	0.52	0.38	0.45	0.068
9	0.51	0.38	0.45	0.065
10	0.64	0.45	0.54	0.098
<b>DI-05 Magnesium Point</b>				
1	0.58	0.56	0.57	0.005
2	0.51	0.53	0.52	0.010
3	0.48	0.51	0.50	0.015
4	0.50	0.44	0.47	0.033
5	0.45	0.50	0.47	0.023
6	0.44	0.56	0.50	0.056
7	0.44	0.44	0.44	0.002
8	0.52	0.43	0.48	0.041
9	0.46	0.46	0.46	0.000
10	0.51	0.37	0.44	0.073

<b>DI-05 Phosphorous Point</b>				
1	12.91	12.27	12.59	0.323
2	13.16	12.88	13.02	0.140
3	14.29	14.51	14.40	0.107
4	14.40	13.32	13.86	0.540
5	13.41	13.49	13.45	0.036
6	13.63	13.98	13.81	0.176
7	14.03	14.30	14.17	0.132
8	13.97	14.56	14.27	0.295
9	14.13	15.19	14.66	0.527
10	13.15	15.15	14.15	1.002
<b>DI-05 Chlorine Point</b>				
1	0.03	0.02	0.03	0.005
2	0.03	0.02	0.03	0.006
3	0.03	0.04	0.04	0.003
4	0.04	0.01	0.03	0.015
5	0.04	0.03	0.03	0.006
6	0.03	0.04	0.03	0.008
7	0.05	0.04	0.04	0.006
8	0.04	0.05	0.05	0.003
9	0.04	0.05	0.04	0.007
10	0.05	0.05	0.05	0.001
<b>DI-05 Potassium Point</b>				
1	0.01	0.01	0.01	0.003
2	0.00	0.01	0.01	0.002
3	0.02	0.01	0.01	0.005
4	0.01	0.01	0.01	0.000
5	0.02	0.01	0.02	0.002
6	0.00	0.01	0.01	0.006
7	0.01	0.02	0.01	0.006
8	0.01	0.02	0.01	0.003
9	0.01	0.02	0.01	0.004
10	0.00	0.01	0.01	0.004
<b>DI-05 Calcium Point</b>				
1	26.38	24.60	25.49	0.890
2	27.00	24.40	25.70	1.298
3	30.70	28.80	29.75	0.947
4	30.14	25.42	27.78	2.360
5	26.59	26.25	26.42	0.166
6	27.34	27.70	27.52	0.182
7	28.42	28.78	28.60	0.181
8	27.18	29.44	28.31	1.131
9	27.31	31.32	29.31	2.006
10	24.99	30.91	27.95	2.962

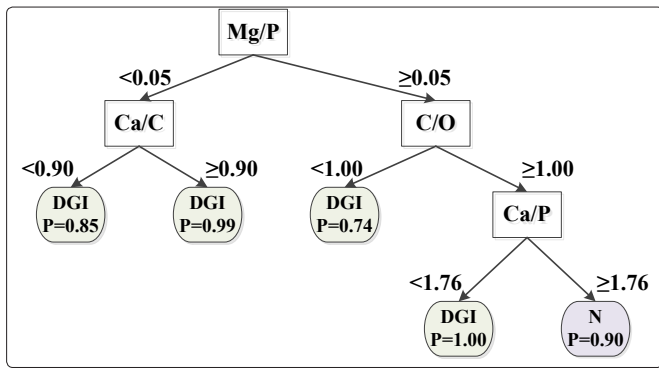


Line	A	B	C	Mean	SD
<b>DI-06 Carbon Point (MV=missing value)</b>					
1	MV	27.88	26.79	27.33	0.543
2	27.37	25.04	25.53	25.98	1.001
3	24.80	24.37	24.31	24.49	0.220
4	23.63	22.98	23.10	23.23	0.281
5	23.17	23.11	24.17	23.49	0.488
6	22.75	22.70	23.41	22.95	0.323
7	22.10	23.12	26.79	24.00	2.015
8	21.82	22.54	26.24	23.53	1.936
9	33.32	23.66	23.25	26.74	4.654
10	MV	25.22	26.36	25.79	0.572
<b>DI-06 Oxygen Point (MV=missing value)</b>					
1	MV	33.79	35.33	34.56	0.772
2	31.75	35.19	35.52	34.16	1.704
3	32.52	35.87	36.44	34.94	1.728
4	36.33	37.64	37.59	37.19	0.607
5	36.80	38.02	36.64	37.16	0.616
6	37.18	37.99	36.07	37.08	0.787
7	37.50	37.08	34.07	36.21	1.530
8	37.83	37.64	32.31	35.93	2.560
9	31.07	35.41	33.49	33.32	1.776
10	MV	34.33	30.83	32.58	1.750
<b>DI-06 Sodium Point (MV=missing value)</b>					
1	MV	0.43	0.45	0.44	0.006
2	0.37	0.47	0.44	0.43	0.042
3	0.42	0.56	0.56	0.51	0.066
4	0.51	0.61	0.57	0.56	0.039
5	0.54	0.57	0.53	0.55	0.015
6	0.56	0.52	0.53	0.54	0.016
7	0.47	0.54	0.45	0.49	0.040
8	0.35	0.45	0.43	0.41	0.043
9	0.27	0.40	0.41	0.36	0.064
10	MV	0.46	0.33	0.40	0.064
<b>DI-06 Magnesium Point (MV=missing value)</b>					
1	MV	0.68	0.75	0.71	0.037
2	0.55	0.62	0.61	0.59	0.030
3	0.52	0.62	0.59	0.57	0.041
4	0.55	0.54	0.35	0.48	0.094
5	0.53	0.35	0.38	0.42	0.080
6	0.44	0.32	0.76	0.51	0.185
7	0.35	0.37	0.42	0.38	0.030
8	0.32	0.47	0.53	0.44	0.086
9	0.24	0.36	0.86	0.49	0.270
10	MV	0.40	0.42	0.41	0.009
<b>DI-06 Phosphorous Point (MV=missing value)</b>					
1	MV	12.66	12.43	12.55	0.115

2	13.22	13.34	13.27	13.27	0.051
3	14.09	13.42	13.44	13.65	0.309
4	13.54	13.32	13.19	13.35	0.141
5	13.57	13.05	13.07	13.23	0.243
6	13.43	13.13	13.92	13.49	0.327
7	13.47	13.27	12.94	13.23	0.221
8	13.34	13.40	13.80	13.51	0.207
9	11.64	13.55	14.76	13.31	1.283
10	MV	13.27	14.00	13.63	0.363
<b>DI-06 Chlorine Point (MV=missing value)</b>					
1	MV	0.02	0.03	0.03	0.004
2	0.04	0.03	0.02	0.03	0.006
3	0.03	0.03	0.04	0.04	0.006
4	0.03	0.05	0.04	0.04	0.007
5	0.05	0.05	0.05	0.05	0.004
6	0.05	0.07	0.05	0.06	0.007
7	0.09	0.08	0.10	0.09	0.007
8	0.09	0.07	0.10	0.09	0.012
9	0.24	0.08	0.07	0.13	0.075
10	MV	0.10	0.11	0.10	0.006
<b>DI-06 Potassium Point (MV=missing value)</b>					
1	MV	0.01	0.01	0.01	0.003
2	0.01	0.02	0.01	0.01	0.003
3	0.00	0.00	0.01	0.01	0.005
4	0.01	0.01	0.00	0.01	0.004
5	0.02	0.01	0.02	0.02	0.006
6	0.00	0.01	0.02	0.01	0.008
7	0.01	0.01	0.01	0.01	0.002
8	0.00	0.02	0.01	0.01	0.008
9	0.01	0.01	0.02	0.01	0.006
10	MV	0.00	0.02	0.01	0.010
<b>DI-06 Calcium Point (MV=missing value)</b>					
1	MV	24.53	24.21	24.37	0.164
2	26.70	25.29	24.60	25.53	0.874
3	27.62	25.13	24.60	25.78	1.317
4	25.40	24.84	25.15	25.13	0.229
5	25.31	24.83	25.13	25.09	0.198
6	25.59	25.26	25.23	25.36	0.163
7	26.01	25.53	25.23	25.59	0.323
8	26.25	25.42	26.58	26.08	0.487
9	23.22	26.52	27.14	25.62	1.721
10	MV	26.22	27.92	27.07	0.853

Line	A	B	Mean	SD
<b>DI-07 Carbon Point</b>				
1	24.55	25.62	25.08	0.533
2	25.11	26.84	25.97	0.867
3	30.47	27.79	29.13	1.343
4	28.99	26.94	27.97	1.023
5	27.81	28.54	28.18	0.364
6	29.38	28.73	29.05	0.328
7	29.54	27.96	28.75	0.791
8	29.11	26.99	28.05	1.063
9	29.49	24.19	26.84	2.652
10	30.54	24.85	27.70	2.845
<b>DI-07 Oxygen Point</b>				
1	33.40	32.86	33.13	0.268
2	33.73	33.36	33.55	0.184
3	32.79	33.67	33.23	0.441
4	31.49	30.48	30.98	0.503
5	31.79	30.73	31.26	0.531
6	30.49	31.44	30.96	0.472
7	29.28	31.80	30.54	1.256
8	31.66	33.87	32.76	1.104
9	31.09	33.87	32.48	1.388
10	31.31	34.77	33.04	1.732
<b>DI-07 Sodium Point</b>				
1	0.47	0.51	0.49	0.019
2	0.51	0.50	0.51	0.007
3	0.49	0.64	0.56	0.077
4	0.55	0.83	0.69	0.138
5	0.75	0.59	0.67	0.078
6	0.60	0.62	0.61	0.009
7	0.95	0.56	0.76	0.190
8	0.70	1.04	0.87	0.168
9	0.77	0.36	0.57	0.204
10	1.20	0.33	0.76	0.434
<b>DI-07 Magnesium Point</b>				
1	0.83	0.79	0.81	0.018
2	0.83	0.72	0.78	0.054
3	0.69	0.64	0.67	0.025
4	0.69	0.61	0.65	0.038
5	0.59	0.73	0.66	0.070
6	0.66	0.73	0.70	0.033
7	0.66	0.80	0.73	0.073
8	0.65	0.36	0.50	0.148
9	0.61	0.57	0.59	0.019
10	0.28	0.62	0.45	0.170
<b>DI-07 Phosphorous Point</b>				
1	13.47	13.36	13.41	0.057

2	13.56	13.03	13.29	0.268
3	12.14	12.62	12.38	0.241
4	12.94	13.76	13.35	0.410
5	13.17	13.21	13.19	0.021
6	13.07	12.97	13.02	0.048
7	13.34	13.15	13.24	0.096
8	12.82	12.67	12.74	0.075
9	12.87	13.78	13.32	0.455
10	12.47	13.59	13.03	0.560
<b>DI-07 Chlorine Point</b>				
1	0.02	0.02	0.02	0.005
2	0.03	0.03	0.03	0.002
3	0.04	0.03	0.03	0.002
4	0.02	0.04	0.03	0.011
5	0.03	0.03	0.03	0.001
6	0.05	0.02	0.03	0.016
7	0.03	0.03	0.03	0.001
8	0.06	0.03	0.04	0.015
9	0.03	0.03	0.03	0.001
10	0.07	0.01	0.04	0.031
<b>DI-07 Potassium Point</b>				
1	0.02	0.01	0.01	0.006
2	0.00	0.01	0.01	0.002
3	0.01	0.00	0.01	0.005
4	0.01	0.01	0.01	0.002
5	0.01	0.01	0.01	0.002
6	0.01	0.01	0.01	0.000
7	0.02	0.02	0.02	0.004
8	0.02	0.01	0.02	0.004
9	0.02	0.00	0.01	0.007
10	0.00	0.00	0.00	0.000
<b>DI-07 Calcium Point</b>				
1	27.24	26.84	27.04	0.199
2	26.23	25.51	25.87	0.356
3	23.37	24.60	23.99	0.617
4	25.30	27.32	26.31	1.007
5	25.86	26.16	26.01	0.152
6	25.74	25.50	25.62	0.122
7	26.18	25.68	25.93	0.247
8	24.98	25.05	25.01	0.033
9	25.12	27.20	26.16	1.041
10	24.13	25.82	24.98	0.847



Knowledge tree induced in Xpert Rule Analyser after 0.1% statistical pruning using the XRMA values for the ratios Ca/P, Ca/C, Mg/P, Mg/Ca and C/O in normal dentine and dentine in teeth from patients diagnosed with Dentinogenesis Imperfecta type II. Normal dentine (N) and dentine from Dentinogenesis Imperfecta teeth (DGI) were set as discrete outcome values in the analysis. The values at the straight arrows show the break points for respective ratio. In the rounded outcome end nodes, the probability (P=) for correctly classified examples is given.

The mean values and standard deviations for intensity corrections from the XRMA analyses of dentine in normal primary teeth and

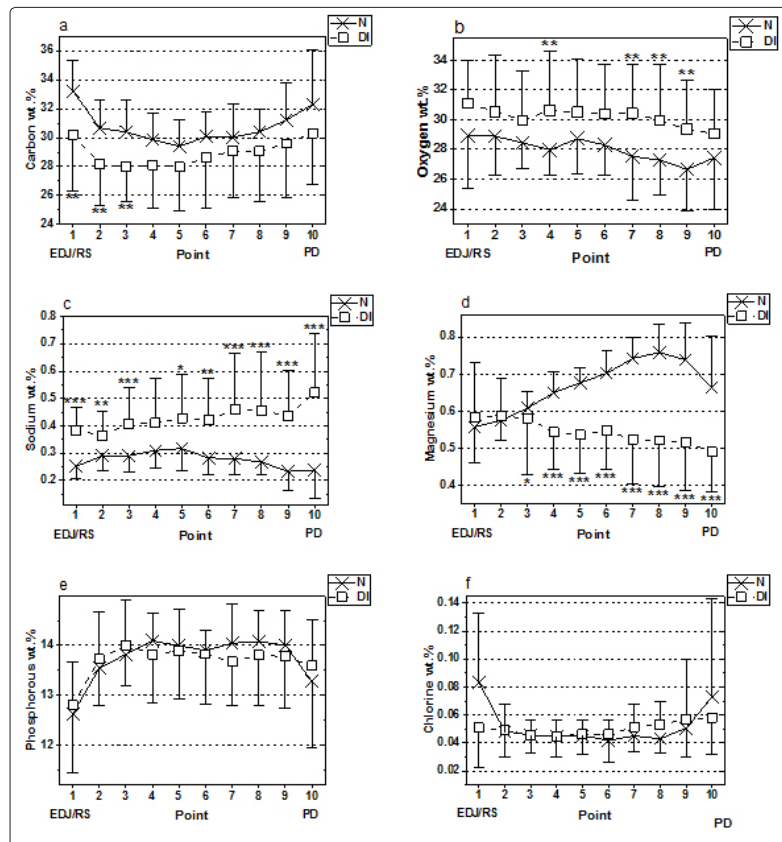
in DI-dentine for the elements C, O, Na, Mg, P, Cl, K and Ca are given in Table 2.

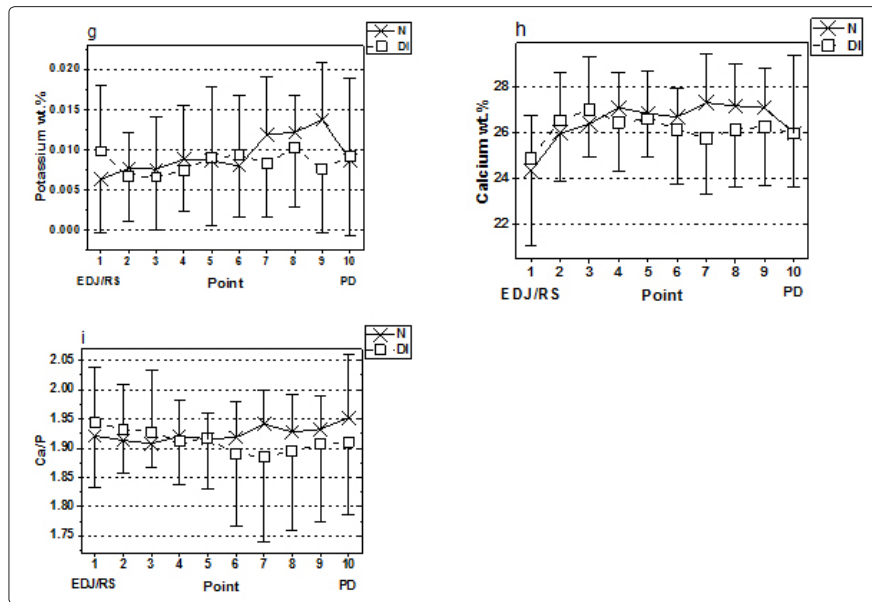
**Table 2:** Mean values (Mean) and standard deviations (SD) for intensity corrections from XRMA analyses of dentine in normal sound primary teeth (N) and in dentine in primary teeth from patients diagnosed with Dentinogenesis Imperfecta type II (DI) for the elements C, O, Na, Mg, P, Cl, K and Ca. Intensity corrections compensate for the matrix effects of the analysed sample and are calculated from the element composition. The apparent concentrations are divided by the intensity corrections to produce the true concentrations

	N		DI	
	Mean	SD	Mean	SD
C	0.21	0.014	0.21	0.029
O	0.17	0.011	0.18	0.020
Na	0.67	0.007	0.66	0.015
Mg	0.66	0.006	0.66	0.013
P	0.96	0.007	0.96	0.014
Cl	1.00	0.004	1.01	0.008
K	1.02	0.007	1.02	0.015
Ca	0.92	0.0023	0.92	0.006

### The Elements C, O, Na, Mg, P, Cl, K, Ca and the Ratio Ca/P (Figure 3a-i)

The concentration profiles for the elements C, O, P, Cl, K and Ca were parallel to each other with only minor differences between normal and DI-dentine.





**Figure 3a-i:** Semi-quantitative mean values and standard deviations for C, O, Na, Mg, P, Cl, K, Ca and the ratio Ca/P from XRMA measurements in dentine from normal primary teeth (N) and from primary teeth diagnosed with Dentinogenesis Imperfecta type II (DI). The measurements are carried out from the enamel-dentine junction/root surface (EDJ/RS) at Point 1 toward the pulpal dentine area (PD) at Point 10 (The statistical analysis is carried out using the Mann-Whitney U test for the values in each point;  $*=p<0.05$ ;  $**=p<0.01$ ;  $***=p<0.001$ .)

The C values were highest close to the enamel-dentine junction/root surface (EDJ/RS) followed by a decrease to approximately the middle part of the dentine and the increased toward the pulpal dentine (PD) area. The C values were lower in DI-dentine and statistically significantly lower values were found in the 3 points closest to EDJ/RS.

The O values decreased from the EDJ/RS toward the PD, the values being higher in DI-dentine. In the points 4 and 7-9 the values were significantly higher in DI-dentine.

In normal dentine the Na values increased from the EDJ/RS toward the mid dentine and then decreased toward the PD. The Na profile in DI-dentine increased from the EDJ/RS toward the PD and had higher values compared with normal dentine. All values for Na in DI-dentine were significantly higher compared with normal dentine with the exception for point 4.

In normal dentine the Mg concentration profile increased from the EDJ/RS toward the PD, which was in contrast to what was found in DI-dentine where the Mg decreased from the EDJ/RS. The Mg values in DI-dentine from point 3 and inwards were significantly lower compared with normal dentine. In the P concentration profile the lowest values were found at the EDJ/RS with a rapid increase

till point 3 and remained flat with a decrease close to the PD. There was no difference in the concentration profiles for P in normal and DI-dentine.

The Cl profile in normal and DI-dentine was at the same level and increased slightly toward the PD.

The K profile increased from the EDJ/RS toward the PD with approximately the same levels in normal and DI-dentine.

For Ca the concentration profiles were lowest at the EDJ/RS and increased till point 4 followed by slight decrease toward the PD. In the inner half of the dentine DI-dentine had lower values.

The concentration profile for the ratio Ca/P in normal and DI-dentine were parallel to each other. The ratio Ca/P had almost the same value in all measurement points with only a minor difference between normal and DI-dentine.

### Inductive Analyses

The full results of the inductive analyses before and after 0.1% statistical pruning for the different elements are given in Table 3 and Figure 4. The break point values were calculated by Xpert Rule Analyser.

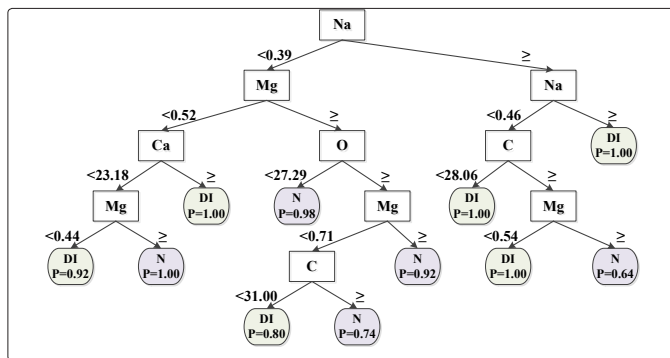
**Table 3:** Results from the inductive analyses before pruning and after 0.1% statistical pruning of XRMA measurements in normal dentine and Dentinogenesis Imperfecta dentine showing the location (Level) in the induced knowledge tree for the elements C, O, Na, Mg, P, Cl, K and Ca and the outcome values normal dentine (N) and Dentinogenesis Imperfecta dentine (DI) with corresponding probability values within brackets. The outcome results are given for training set and the test set of data (Redundant element=not appearing in the knowledge tree)

No pruning (number of end nodes=26)		
Level	Element	Outcome (probability)
1	Na	
2	Na; Mg	
3	C; O; Ca	DI (1.00)
4	C; Mg	DI (1.00); DI (1.00)
5	C; O; Cl	DI (1.00); N (1.00); N (0.75); N (1.00)
6	C; Na	DI (0.75); DI (1.00); DI (0.71); N (1.00); N (1.00)
7	O	DI (1.00); DI (0.75); N (1.00); N (1.00)
8	C; O; Na; P	
9	Mg	DI (0.75); DI (1.00); DI (1.00); N (1.00); N (0.75); N (1.00); N (0.75)
10	P	DI (1.00)
11		DI (0.75); N (0.75)

After pruning (number of end nodes=11)	
Element	Outcome (probability)
Na	
Na; Mg	
C; O; Ca	DI (1.00)
Mg	DI (1.00); DI (1.00); N (0.98)
C	DI (0.92); DI (1.00); N (1.00); N (0.92); N (0.64)
	DI (0.80); N (0.74)

P; Cl; K		
% correctly Training		
Training	93.8	classified Test
N	90.8	89.9
Overall	92.8	75.0

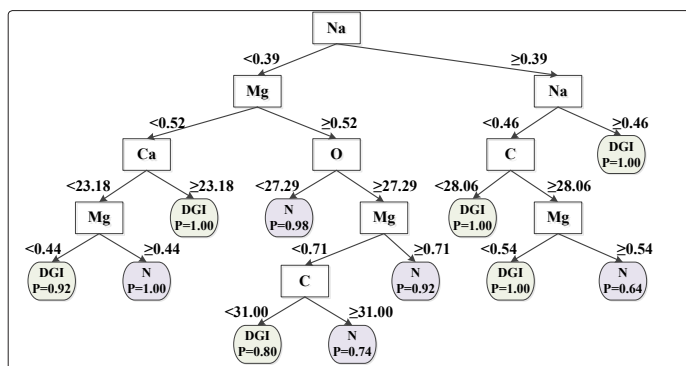
Redundant elements: K		
Outcome results		
	% correctly Training	classified Test
DI	98.4	94.6
N	95.0	75.0
Overall	97.3	88.3



**Figure 4:** Knowledge tree induced in Xpert Rule Analyser after 0.1% statistical pruning using the XRMA values for C, O, Na, Mg, P, Cl, K and Ca in normal dentine and dentine in teeth from patients diagnosed with Dentinogenesis Imperfecta type II. Normal dentine (N) and dentine from Dentinogenesis Imperfecta type II (DI) were set as discrete outcome values in the analysis. The values at the straight arrows show the break points for respective element. In order to make the knowledge tree more feasible to read only the values of the left branch (<) are shown, the right branches having the same values after the sign ≥. In the rounded outcome end nodes, the probability (P=) for correctly classified examples is given

At the top level of both knowledge trees was Na found and at the second level Na and Mg, thus being the two most important elements for discriminating normal dentine from DI-dentine. K did not appear in the knowledge tree, thus being redundant in the analysis. Before pruning the number of end nodes was 26 compared with 11 after statistical pruning of 0.1%. The percentage correctly classified outcome values “DI-dentine” and “normal dentine” before pruning were 98.4% and 95.0%, respectively. The corresponding values for the test set in which the induced rule was verified was lower (94.6% and 75.0%).

In order to make the results more explicit only the knowledge tree after 0.1% statistical pruning is shown in Figure 5. The first three levels of the knowledge tree contained the same elements before and after pruning. In the pruned knowledge tree P, Cl and K did not appear, thus being redundant. The percentage correctly classified outcome values “DI-dentine” and “normal dentine” after pruning were 93.8% and 90.8%, respectively. The corresponding values for the test set in which the induced rule was verified was lower (89.9% and 74.2%).



**Figure 5:** Knowledge tree induced in Xpert Rule Analyser after 0.1% statistical pruning using the XRMA values for C, O, Na, Mg, P, Cl, K and Ca in normal dentine and dentine in teeth from patients diagnosed with Dentinogenesis Imperfecta type II. Normal dentine (N) and dentine from Dentinogenesis Imperfecta type II (DGI) were set as discrete outcome values in the analysis. The values at the straight arrows show the break points for respective element. In the rounded outcome end nodes, the probability (P=) for correctly classified examples is given

## Discussion

The present study has shown that the concentration profiles for C, O, Na and Mg measured by XRMA in dentin from primary teeth diagnosed with dentinogenesis imperfecta (DI) differ from normal primary dentine. The most marked differences were found for Na and Mg which was confirmed in the inductive analysis.

XRMA has proven to be a useful method for analysis of some inorganic elements in dental hard tissues with control of the morphological location of the measurements [23,24]. Even if the values of the measurements are to be considered as semi-quantitative, comparisons between different locations and different samples can be made when the parameters for the XRMA analysis are well controlled. Inductive analysis seen as a tool for pattern recognition is a complement to more traditional statistical methods and has been used in several areas of research [24-26]. The evolved rules are induced using a randomized sample of 50% of the examples, these rules can then be evaluated in the verifying option of Xpert Rule Analyser against the remaining examples. The hierarchic presentation and the possibility for verifying the knowledge tree makes the inductive analysis a powerful tool in research.

The carbon values for DI-dentine were lower than found in normal dentine. In XRMA analysis carbon is a sum of what is located in the inorganic and organic matrix and may possibly reflect lower values of carbonates in the inorganic phase of the DI-dentine. Since the water content in DI-dentine is higher than in normal dentine it has been suggested that it may be related to water bound to the crystallite lattice in the inorganic phase, which could explain the increase of oxygen seen in DI-dentine [21].

The lower values of Mg in DI-dentine compared with normal dentine are in concordance with electron microprobe analyses of DI-dentine [21]. It has been shown that in rat developing dentine that the ratio Mg/P from the peripheral dentine toward the central dentine increased [27]. This in contrast to what was found in the present study with lower values of Mg decreasing from the enamel-dentine junction and inwards compared with normal dentine, possibly may be

related to an impaired function of odontoblasts in the mineralization of dentine. In a study of bovine peritubular dentine it was shown that Mg<sup>2+</sup> and K<sup>+</sup> to a high extent are associated with the organic matrix, however, in the present study it was not possible to discriminate between peritubular and intertubular dentine [28].

It has previously been shown that an increase in the content of Mg may lead to a decrease in the Ca/P ratio, however, in the present study there were no differences found in the Ca/P ratio in DI-dentine and normal dentine [29]. The values for P, Ca and the ratio Ca/P did not differ between DI and normal dentine which is in contrast to what was found in a previous report [21]. The relatively flat concentration profiles of P and Ca decreasing towards the pulpal dentine is in line with previous studies [30,31]. The Ca/P ratio in the dentine in primary normal teeth and primary teeth from patients with hypophosphatemic rickets, showed only minor differences [32]. The ratio Ca/P is often presented in the literature as an indicator of the presence of hydroxyapatite in dental hard tissues. However, the Ca/P ratio is not regarded to be useful as an indicator of the calcium phosphate phase [33].

A previous study has shown that Na, Mg, P and Ca precipitated together in the formation of a homogenous carbonate apatite [34]. In the present study the concentration profile of Na was inverted to what was found for Mg. The high values of Na are typical for extra cellular tissue in predentine and may reflect an impaired mineralization of DI-dentine [35]. In the inductive analysis Na was found at top node, thus being the most important element for discriminating DI-dentine from normal dentine, however, the underlying factors is at present not known.

## Conclusion

This study has shown that dentine in primary teeth from patients diagnosed with Dentinogenesis Imperfecta type II analysed with XRMA have lower values of C and Mg and higher values of O and Na compared with normal primary dentine. Further the inductive analysis revealed that Na was the most important element for discriminating DI-dentine from normal primary dentine.

## References

- Shields ED, Bixler D, el-Kafrawy AM (1973) A proposed classification for heritable human dentine defects with a description of a new entity. *Arch Oral Biol* 18: 543-553.
- Seow WK (2014) Developmental defects of enamel and dentine: challenges for basic science research and clinical management. *Aust Dent J* 59: 143-154.
- Pallos D, Hart PS, Cortelli JR, Vian S, Wright JT, et al. (2001) Novel COL1A1 mutation (G559C) [correction of G599C] associated with mild osteogenesis imperfecta and dentinogenesis imperfecta. *Arch Oral Biol* 46: 459-470.
- Kim JW, Nam SH, Jang KT, Lee SH, Kim CC, et al. (2004) A novel splice acceptor mutation in the DSPP gene causing dentinogenesis imperfecta type II. *Hum Genet* 115: 248-254.
- Kim JW, Simmer JP (2007) Hereditary dentin defects. *J Dent Res* 8: 392-399.
- Barron MJ, McDonnell ST, Mackie I, Dixon MJ (2008) Hereditary dentine disorders: dentinogenesis imperfecta and dentine dysplasia. *Orphanet J Rare Dis* 3: 31.
- Beattie ML, Kim JW, Gong SG, Murdoch-Kinch CA, Simmer JP, et al. (2006) Phenotypic variation in dentinogenesis imperfecta/dentin dysplasia linked to 4q21. *J Dent Res* 85: 329-333.

8. Hart PS, Hart TC (2007) Disorders of human dentin. *Cells Tissues Organs* 186: 70-77.
9. Bailleul-Forestier I, Molla M, Verloes A, Berdal A (2008) The genetic basis of inherited anomalies of the teeth. Part 1: clinical and molecular aspects of non-syndromic dental disorders. *Eur J Med Genet* 51: 273-291.
10. Bailleul-Forestier I, Berdal A, Vinckier F, de Ravel T, Fryns JP, et al. (2008) The genetic basis of inherited anomalies of the teeth. Part 2: syndromes with significant dental involvement. *Eur J Med Genet* 51: 383-408.
11. Levin LS, Leaf SH, Jelmini RJ, Rose JJ, Rosenbaum KN (1983) Dentinogenesis imperfecta in the Brandywine isolate (DI type III): clinical, radiologic, and scanning electron microscopic studies of the dentition. *Oral Surg Oral Med Oral Path* 56: 267-274.
12. McKnight DA, Simmer JP, Hart PS, Hart TC, Fisher LW (2008) Overlapping DSPP mutations cause dentin dysplasia and dentinogenesis imperfecta. *J Dent Res* 87: 1108-1111.
13. Leal CT, Martins LD, Verli FD, de Souza MA, Ramos-Jorge ML (2010) Case report: Clinical, histological and ultrastructural characterization of type II dentinogenesis imperfecta. *Eur Arch Paediatr Dent* 11: 306-309.
14. Waltimo J (1994) Hyperfibers and vesicles in dentin matrix in dentinogenesis imperfecta (DI) associated with osteogenesis imperfecta (OI). *J Oral Pathol Med* 23: 389-393.
15. Gallusi G, Libonati A, Campanella V (2006) SEM-morphology in dentinogenesis imperfecta type II: microscopic anatomy and efficacy of a dentine bonding system. *Eur J Paediatr Dent* 7: 9-17.
16. Wiczorek A, Loster J (2013) Dentinogenesis imperfecta type II: ultrastructure of teeth in sagittal sections. *Folia Histochem Cytobiol* 51: 244-247.
17. Waltimo J (1994) Hyperfibers and vesicles in dentin matrix in dentinogenesis imperfecta (DI) associated with osteogenesis imperfecta (OI). *J Oral Pathol Med* 23: 389-393.
18. Lindau B, Dietz W, Lundgren T, Storhaug K, Norén JG (1999) Discrimination of morphological findings in dentine from osteogenesis imperfecta patients using combinations of polarized light microscopy, microradiography and scanning electron microscopy. *Int J Paediatr Dent* 9: 253-261.
19. Davis GR, Fearn JM, Sabel N, Norén JG (2015) Microscopic study of dental hard tissues in primary teeth with Dentinogenesis Imperfecta Type II: Correlation of 3D imaging using X-ray microtomography and polarising microscopy. *Arch Oral Biol* 60: 1013-1020.
20. Kinney JH, Pople JA, Driessen CH, Breunig TM, Marshall GW, et al. (2001) Intrafibrillar mineral may be absent in dentinogenesis imperfecta type II (DI-II). *J Dent Res* 80: 1555-1559.
21. Kerebel B, Daculsi G, Menanteau J, Kerebel LM (1981) The inorganic phase in dentinogenesis imperfecta. *J Dent Res* 60: 1655-1660.
22. Wiczorek A, Loster J (2012) Analysis of the mineral composition of permanent teeth in dentinogenesis imperfecta type II. *J Stoma* 65: 404-410.
23. Melin L, Norén JG, Taube F, Cornell DH (2014) Evaluation of X-ray microanalysis for characterization of dental enamel. *Microsc Microanal* 20: 257-267.
24. Melin L, Lundgren J, Malmberg P, Norén JG, Taube F, et al. (2015) XRMA and ToF-SIMS Analysis of normal and hypomineralized enamel. *Microsc Microanal* 21: 407-421.
25. Nilsson T, Lundgren T, Odelius H, Jönsson U, Sillén R, et al. (1998) Differences in co-variation of inorganic elements in the bulk and surface of human deciduous enamel: an induction analysis study. *Connect Tissue Res* 38: 81-89.
26. Klingberg G, Sillén R, Norén JG (1999) Machine learning methods applied on dental fear and behavior management problems in children. *Acta Odontol Scand* 57: 207-215.
27. Steinfort J, Driessens FC, Heijligers HJ, Beertsen W (1991) The distribution of magnesium in developing rat incisor dentin. *J Dent Res* 70: 187-191.
28. Gotliv BA, Robach JS, Veis A (2006) The composition and structure of bovine peritubular dentin: mapping by time of flight secondary ion mass spectroscopy. *J Struct Biol* 156: 320-333.
29. Stratmann U, Schaarschmidt K, Wiesmann HP, Plate U, Höhling HJ (1996) Mineralization during matrix-vesicle-mediated mantle dentine formation in molars of albino rats: a microanalytical and ultrastructural study. *Cell Tissue Res* 284: 223-230.
30. Tøtdal B, Hals E (1985) Electron probe study of human and red deer cementum and root dentin. *Scand J Dent Res* 93: 4-12.
31. Hals E, Tveit AB, Tøtdal B (1988) X-ray microanalysis of dentin: a review. *Scanning Microsc* 2: 357-369.
32. Boukpepsi T, Septier D, Bagga S, Garabedian M, Goldberg M, et al. (2006) Dentin alteration of deciduous teeth in human hypophosphatemic rickets. *Calcif Tissue Int* 79: 294-300.
33. Drouet C (2013) Apatite formation: why it may not work as planned, and how to conclusively identify apatite compounds. *Biomed Res Int* 2013: 490946.
34. Eidelman N, Boyde A, Bushby AJ, Howell PG, Sun J, et al. (2009) Microstructure and mineral composition of dystrophic calcification associated with the idiopathic inflammatory myopathies. *Arthritis Res Ther* 11: R159.
35. Wiesmann HP, Höhling HJ, Zierold K, Barckhaus R (1995) Elemental distributions in predentine associated with dentine mineralization in rat incisor. *Connect Tissue Res* 33: 179-184.

**Copyright:** ©2018 Jorgen G Noren, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.