DEVELOPMENT OF A PREDICTION MODEL FOR HEMP (*CANNABIS SATIVA* L.) CELL WALL COMPOSITION USING NEAR-INFRARED SPECTROSCOPY (NIRS) AND BIOCHEMICAL ANALYSIS

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Abstract

Hemp (*Cannabis sativa* L.) biomass can be used for a wide range of renewable materials and bio-composites. Hemp fibres can be used as a raw materials for biomaterias such as paper and textile. Appart from its versatile biomass hemp is an environmental friendly crop which needs less irrigation and agrochemical demands than other fibre crops. High fibre quality is a desirable trait and generally defined by a high cellulose content, low degree of lignification and a reduced number of cross links between the pectin and the structural components of the cell wall regarding chemical composition. In this project, 124 hemp accessions were collected and grown in three different locations in Italy, France and the Netherlands in three blocks per location. After that, hemp stems were harvested and grinded to study the biochemical composition which can be determined by biochemical analysis or by prediction the trait using a model. From the more than 1000 stem samples, about 100 samples were selected to develop models for the Near-Infrared Spectroscopy (NIRS) in order to predict the percentage of different cell wall components in the remaining samples. In this experiment, two statistical models were developed to predict the percentage of cell wall components. The predicted data showed that there are differences in cell wall components among accessions and within the same accession grown in different locations. The data also showed that the percentages of neutral detergent fibre (NDF) and acid detergent fibre (ADF) were higher in the north than in the south part of Europe. Afterwards, further experiment should be done to determine the percentage of every cell wall component. These results will allow the identification of the best accessions, with the highest fibre quality, for each locations.

Key words: hemp, fibre quality, NIRS, biochemical analysis, cell wall component

1 Introduction

1.1 The hemp plant and its history

There are three main types of *Cannabis* in the *Cannabaceae* family: *Cannabis sativa, Cannabis indica* and *Cannabis ruderalis* (Anwar et al., 2006). All *Cannabis* species in nature are dioecious and are reported to have 2n=20 chromosomes (Mandolino et al., 1999). Through breeding monoecious cultivars have been developed as well (Mandolino et al., 1999). Monoecious hemp is useful specially when hemp is cultured as a multipurpose crop, when both seed production and stem harvesting are performed (Bócsa, 1994). Hemp (*Cannabis sativa* L.), an annual herbaceous plant, is one of the most ancient cultivated crops (Mandolino et al., 1999; Oomah et al., 2002; Struik et al., 2000). It is considered to be native to Western and Central Asia (Russia, China India, Pakistan and Iran) (Anwar et al., 2006). Traditionally, hemp was grown for its long and strong bast fibres and seeds, then the cultivation of hemp was dramatically reduced for decades because of the use of cannabis as a narcotic, only in eastern Europe, the former Soviet-Union and China the hemp industry survived (Meijer, 1995). Today hemp is cultivated on 10,000 to 15,000 ha in the European Union including in the UK, France, the Netherlands, Germany, Spain and Italy (Carus et al., 2013).

Hemp is an attractive crop for sustainable fibre production, it has high yield, fits well in crop rotation schemes, can improve soil structure and has low requirements of fertiliser (Du Bois, 1982; Hanson, 1980; Toonen et al., 2004). Furthermore, weed growth can be suppressed during hemp growth, and hemp is virtually free from pests so it can be grown without pesticides (Toonen et al., 2004; Van der Werf, 2004). However, hemp is not entirely a disease-free crop, for example, it can be infected by the fungus *Botrytis cinerea* under severe wet weather conditions (Van der Werf et al., 1995). Hemp also can have problems because of low temperature, poor soil structure and shortage or excess of water during establishment of the crop (Struik et al., 2000). Compared to cotton, another fibre crop, hemp can be produced in a more sustainable way. Cotton growth causes many negative effects on the environment, such as intensive use of pesticides, high fertiliser and irrigation requirements (Pimentel et al., 1993; Soth et al., 1999).

1.2 Applications of hemp

Hemp is a multi-purpose crop and many parts of the plant can be used (Figure 1). Hemp fibre can be used for paper, rope, fabric, insulation material and bio-composites and it has been widely used in many civilizations for over 6000 years (Beckermann, 2007; Roulac, 1997). Hemp shives are used for construction and animal bedding. Hemp seeds can produce foods with high nutritional value, hemp oil can produce food oil, personal care products and has an excellent and unique fatty acid profile (Carus et al., 2013). Hemp leaves and inflorescence can produce medicine and agro-chemical (reviewed in Salentijn et al., 2014).

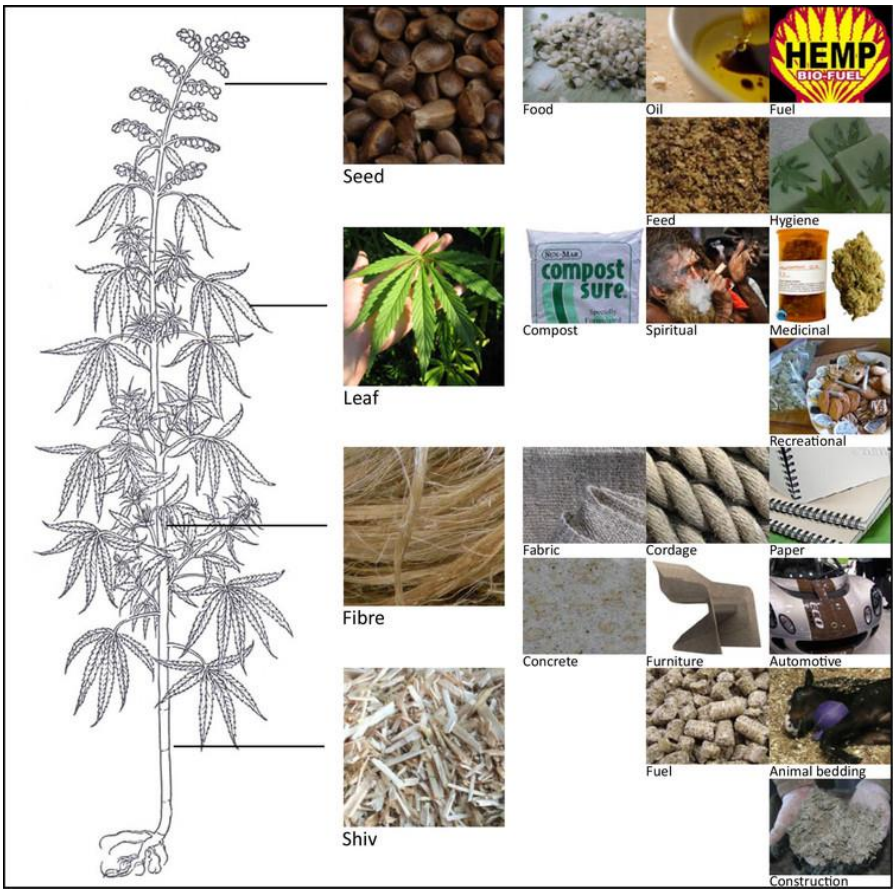

Figure 1 Hemp's constituent parts and their uses (Robinson and Schultes, 1996)

1.3 Hemp cell wall composition and hemp fibre quality

Nowadays, the interest in natural vegetable fibres is increasing. Natural fibres have many advantages like economic viability, enhanced energy recovery, good biodegradability, low density (Dhakal et al., 2007; Le Troedec et al., 2008). However, natural fibres also have some weak points, like their physical and chemical properties are strongly dependent on genotype, harvest and environment (Le Troedec et al., 2008; Van de Weyenberg et al., 2006). Natural fibres can be briefly categorised as woody and non-woody fibres (van den Broeck et al., 2008). Woody fibres are single cells and their properties depend on the type of cells and their functions in the tree (van den Broeck et al., 2008). Non-woody fibres are collections of individual cells called elementary cells and classified according to where these cells are found in the plant (Stevens and Müssig, 2010). Hemp fibre belongs to the non-woody type and it has two types of fibres, the bast fibres and the shives.

Hemp fibre is one of the strongest and stiffest available natural fibres, therefore it has great potential for applications in bio-composite materials (Pickering et al., 2007; Pickering et al., 2005). On the plant level, this is laid down in morphological composition of the hemp stem and development of cell wall during growth (Toonen et al., 2004). The bast fibres are obtained from the inner bark or phloem of the fibre bearing plant and are amongst the strongest and stiffest of all vegetable fibres, because of this, bast fibres are of particular interest composite reinforcement (Figure 2) (reviewed in Hughes, 2012). Bast fibres are collections of one to three dozen phloem elementary fibres about 20mm to 50mm long with a pericyclic form and thick cell walls from 5 to 15 µm (De Meijer, 1994; De Meijer

and Keizer, 1994; Mediavilla et al., 2001; Toonen et al., 2004; Van der Werf et al., 1994b). Elementary fibre consists of a primary and secondary cell wall. The shives or 'woody core' come from the inner part of the stems are derived from the lignin rich xylem tissue and about 0.5-0.6mm long (De Meijer, 1994; De Meijer and Keizer, 1994; Mediavilla et al., 2001; Toonen et al., 2004; Van der Werf et al., 1994b).

Hemp fibres consist of cellulose, lignin, a matrix of polysaccharides including hemicellulose, pectin and proteins. Dupeyre and Vignon, 1998 reported that bast fibres from harvested hemps consist of about 55% cellulose, 16% hemicelluloses, 18% pectin substances and 4% lignin and the shives are rich in lignin (20-50%), less rich in cellulose (35-40%) (Thomsen et al., 2006). There are also some non-structural components including waxes, inorganic salts and nitrogenous substances are consisted in hemp fibres (Dinwoodie, 2000; Sharma and Van Sumere, 1992). In the cell wall, cellulose is mainly formed of highly ordered bundles of cellulose polymers known as microfibrils and embedded in a matrix of other polysaccharides and lignin (reviewed in Hughes, 2012). The microfibrils are arranged helically in three secondary cell wall layers called S1, S2 and S3. The Second layer (S2) strongly influences the axial tensile properties of the fibre. The S1 layer is important in controlling fibre stability in compression by limiting excessive lateral cell expansion and the S3 layer resists hydrostatic pressure within the cell (Booker and Sell, 1998). The winding angle in the S2 layer in hemp bast fibre is usually lower than 10° which means higher strength and stiffness, this seems to be the reason why bast fibres are good choices as composite reinforcement (Mark, 1967; Thygesen et al., 2007).

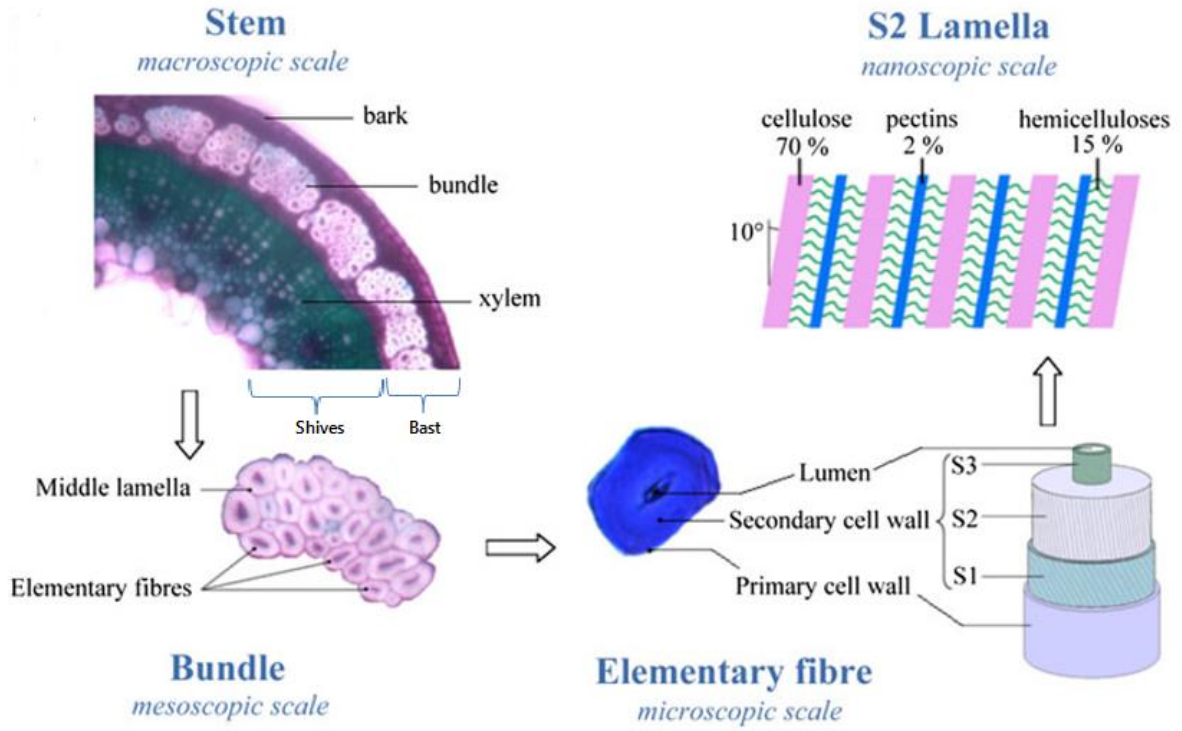

Figure 2 Cross-sections and schematic representations of hemp at different scales, from the stem to the cellulose fibrils (modified from Charlet et al., 2010)

Hemp fibres are interesting natural material for many applications; however, fibre quality is needed to improve. Hemp fibre quality can be determined by chemical composition, fineness, mechanical

and sorption properties (Kostic et al., 2008). Regarding chemical composition, high fibre quality can be defined by high cellulose content, low degree of lignification and reduced number of cross links between the pectin and the structural components of the cell wall (Mandolino and Carboni, 2004). Higher cellulose is needed because it provides strength in cell wall (Lennholm and Blomqvist, 2003). Lignin creates mechanical incrustations in sections of the amorphic cellulose which contributes to fibre lignification (Waśko and Mańkowski, 2004). The high content of lignin increase stiffness, makes fibre more breakable, and reduces its divisibility and spinnability (Nykter et al., 2008; Waśko and Mańkowski, 2004). These features makes the hemp fibre hard to extract and thus reduce hemp fibre quality. Therefore, low degree of lignification is expected in high quality hemp fibre. High fibre quality is also defined by good decortication features which means to separate bast fibres and shives easily (Easson and Molloy, 1996). Pectin is present in the middle lamella between cells of all types and hold the fibres together (Love et al., 1994). To separate bast fibres and shives in hemp stems means to degrade pectin which bind the bast fibres and shives together by retting of hemp stems with minimal damage of fibre. This feature depends on the degradation of pectin which related to hemp fibre quality. To define fibre quality in details, the chemical composition of hemp fibres need to be determined (Van der Werf et al., 1994b).

1.4 Research objective and questions

This minor thesis is part of an EU project, involving 23 Industrial and academic partners, called Multihemp with main focus on 'Genome-wide association mapping for hemp breeding'. The purpose of this project is to phenotype the cell wall composition of 124 hemp accessions using a high-throughput approach. The overall objective of the Multihemp project is 'Multipurpose Hemp for industrial bio-products and biomass is to advance the scientific and technical research needed to consolidate and expand the market of hemp renewable materials'. In this section, we expect to develop a model by using Near-infrared spectroscopy (NIRS) and biochemical analysis to predict the cell wall composition of all the hemp samples cultivated from 2013 in this project without the need to analyse plant by plant.

Therefore, the main goal of this minor thesis is **to develop a prediction model for NIRS and to study the variation of cell wall composition among the hemp accessions.**

Question 1 Is that possible to a model to predict the phenotype of hemp fibre?

Question 2 In which location the accessions show extremes phenotypes?

Question 3 Which accessions with the highest fibre quality in each location?

2 Materials and methodology

2.1 Plant materials and sample preparation

In this project, there are 124 hemp accessions that come from 16 different countries across Europe, China and Canada. This set contains both wild accessions and breeder's materials that were selected according to contrasting behaviour for different traits, including morphological, physical and quality traits. These 124 accessions were grown at three different locations in 2013 – Rovigo (Italy), Chèvrenolles, Neuville-sur-Sarthe (France) and Westerlee (The Netherlands) at 45°N 11°E, 48°N 0.2°E and 53°N 6°E. Each location had a randomized block designed with three blocks. The experimental unit was a plot of 1 m^2 (1.5 m^2 in the French field trial). Hemp accessions were harvested at full flowering and five stems were collected randomly from each plot.

The collected stems were chopped to 2 cm. size pieces by using the chopper machine 'Ets. Rene Pierret'. Then, the chopped stems were put in the oven with 60°C for 1.5 hours. This dried material was grinded using the grinder machine 'Peppink 200AN' by using 1 mm. size filter. Each grinded sample was put in a zipper plastic bag for future analyse. During these procedures, only the same chopper machine, grinder machine and 1 mm. size filter was used to prevent variation caused by using different machines.

2.2 Near-infrared spectroscopy (NIRS) analysis

The Near-Infrared spectroscopy is a technique that provides multi-constituent analysis of almost all matrix (Reich, 2005). It is used in many ecological studies which need chemical analysis of plant and animal tissues (Foley et al., 1998). This technique requires minimum preparation of the samples, the analysis is fast and accurate, many constituents can be analysed at the same time and at a low cost (Batten, 1998; Xu et al., 2013). NIRS analysis is based on vibrational spectroscopy that monitors changes in molecular vibrations intimately associated with changes in molecular structure (Toonen et al., 2004). The NIR spectrum is the sum of absorbance of a number of chemical bonds (Toonen et al., 2004). The most prominent absorption bands occurring in the NIR region are related to overtones and combinations of fundamental vibrations of –CH, -NH, -OH (and -SH) functional groups (Reich, 2005).

NIRS^{TM} DS2500 was used to scan all grinded samples and then, the NIRS spectrum data of these samples were available for further analyse. The next step after that was to obtain laboratory reference values (biochemical data) for all hemp set samples which would be very expensive, so a method was used to select a small set of samples from all hemp set samples for calibration. First of all, the spectrum data of all hemp set samples were extracted by using the software 'Mosaic Solo (NIRS DS2000)' and ranked according to their H distance by using the software 'WinISI Project Manager'. One standardized H distance is the Global H (GH) which shows how different a sample is from the average of all samples (Shenk and Westerhaus, 1991). The spectrum with GH values higher than 3.0 was considered as an outlier. Another one is the minimum standardized H distance - the Neighbourhood H (NH), it is used to control the closeness of neighbouring samples within the dataset and it is the distance to the closest neighbouring sample (Olinger et al., 2001). Then, 101 calibration samples were selected from all the scanned hemp samples and were used to determine their phenotype biochemically.

2.3 Biochemical analysis

The selected calibration samples were biochemically analysed by using the ANKOM technology to determine neutral detergent fibre (NDF) and acid detergent fibre (ADF) (Goering and Van Soest, 1970). The percentage of NDF is the sum of the hemi-cellulose, cellulose, insoluble pectin and lignin parts of the fibre and the ADF percentage is the sum of the lignin and cellulose parts of the fibre.

Three technical replicates were measured for each trait (NAF and ADF) of the calibration samples, in total, 101*3*2=606 filter bags were made. An amount of 0.45-0.5 g. grinded sample was weighed into a 'F57 filter bag' and sealed twice with a heat sealer. Positive control samples and blank samples were also made; the positive control samples were the mix of several samples selected randomly from all hemp set samples. Then, the filter bags were put in 103°C oven over night or at least 5 hours and the dry matter content of the samples was measured. After that, the samples were washed by Neutral Detergent Solution or Acid Detergent Solution with the ANKOM machine 'ANKOM^{2000} fibre analyser'. Using the ANKOM machine, 24 filter bags can be analysed in each round, which means one positive control sample, one blank sample and 22 hemp samples. Quality control was also assessed, in particular differences between different runs. For example, if one positive control had a significant different result compared to the average of the positive controls, this meant that something went wrong in that round and it was better to analyse the samples of that specific round again.

The Neutral Detergent Solution (NDF solution) was made each time by adding 300 g. Sodium dodecyl sulphate (USP), 186.1 g. Ethylenediaminetetraacetic disodium salt (dehydrate), 68.1 g. Sodium borate, 45.6 g. Sodium phosphate dibasic (anhydrous), and 100.0 ml. Triethylene glycol to 10 L. distilled H_20. All the chemicals were added in the fume hood wearing gloves. After this, the pH of the solution was checked, the pH was between 6.9 to 7.1. Then, NDF solution was connected to the Port A of the ANKOM^{2000} fibre analyser and three other components were added at the same time: 20 g. sodium sulphite, 4.0 ml. of Alpha-amylase to the solution directly in the vessel and 8ml Alpha-amylase to 250 ml. of distilled water that were pull into a cup connect to the Port B of ANKOM machine to determine the percentage of NDF (ANKOMTechnology, 2014b).

The Acid Detergent Solution (ADF solution) was made each time by adding 200 g. cetyl trimethylammonium bromide (CTAB) and 266 ml. 98% Sulphuric acid (H_2SO_4) to 10 L. distilled H_2O. As sulphuric acid is a strong acid and can cause severe burns, the ADF solution was prepared in the fume hood and wearing lab coat and gloves. Then, ADF solution were connected to the Port B of ANKOM machine to analyse the percentage of ADF of the samples (ANKOMTechnology, 2014a).

After the NDF or ADF treatment, the washed samples were put in acetone for about 5 minutes and placed in the fume hood to dry. After two hours in the fume hood, the samples were transferred into the oven at 103°C over night and then the dry weight of each sample was measured.

In order to check whether the models developed from the calibration samples were reliable or not, 25 validation samples from all hemp set samples (except the calibration samples used to develop the models) were selected randomly. The procedures for these validation samples were exactly the same as the ones for the previous calibration samples to measure the %NDF and %ADF as well.

2.4 Data analysis

The dry matter content data was loaded into the Excel file. The weight of empty filter bags, filter bag + sample and dry weight of filter bag + sample were measured. The initial weight of the blank samples and the dry weight were used to correct the weight of the filter bags. Then, the estimated dry weight before ANKOM treatment was calculated. After the ANKOM treatment, the dry weight of the samples was measured. Finally, taking into account all these values the percentage of NDF and ADF was calculated. The standard deviation (SD) and the relative standard deviation (RSD) of the percentage of NDF and ADF were also calculated of each sample using the three replicates. All the samples with RSD higher than 15 were considered not reliable and the measurements of the dry weight were repeated or more technical replicated were included until reach a RSD below 15.

The data of %NDF and %ADF of calibration samples were analysed by using the software 'WinISI Project Manager' to develop one model per each trait. The statistical analysis that performed was the Principal component analysis (PCA). The setting of wavelengths and math treatment was SNV and Detrend (standard one), derivative '1', gap '4', smooth '4' and smooth 2 '1' (the setting of 'smooth' and 'smooth 2' is always '4' and '1'), and H or R measurement, H or R value was '3'. The components used to measure GH was '7' which meant the 98.34% of the variability was explained using 7 components. Normally to develop a consistent model the component number should be between 5 to 10 to explain as much as possible the variation present in the NIR spectrum within a limit. More components can explain more variation within the developed model. However, using many components, the developed model is more specific to the samples that have been used to develop the model instead to predict the new data, using only the NIR spectrum (Olinger et al., 2001). In this case, '7' was a suitable component number used to create the model. Moreover, the model equations for %NDF and %ADF were developed by using the 'Modified PLS' regression method.

The 25 randomly selected samples were used to validate the developed models. To check whether or not the developed models are good models, RSQ values were checked. A model is better when the RSQ value is closer to '1'. Another way to check the quality of the models is to check the standard deviation of the predicted data (S.E.P.). When the S.E.P. is lower than three times the standard deviation of the biochemical data (S.E.L.), it also means that the developed model is a good one. Finally, when the validation samples showed good statistics of the developed model, these ones can be used to predict the %NDF and %ADF of all hemp set samples.

3 Results and discussion

In this experiment, instead of 1116 samples (124 accessions in three locations and three replicates), 1034 samples were scanned by using NIRS because there were some missing samples or samples with low amount. In these 1034 samples, 101 calibration samples were selected and used to develop models of %NDF and %ADF. There were also 25 samples randomly selected from the rest 933 samples to validate the developed models.

After the ANKOM treatments, the results showed that all the calculated RSD of calibration samples and validation samples were much lower than 15. These results mean that the differences between the three technical replicates were not statistical significant. Therefore, the data are reliable and can be used for further experiments. The biochemical data of the %NDF and the %ADF of calibration samples and validation samples can be found in Appendix 1 and Appendix 2.

By using the %NDF and %ADF dry matter results of the model samples, the models to calculate all the samples' %NDF and %ADF dry matter were developed (Table 1).

Table 1 Developed models for %NDF and %ADF by using calibration samples

CONSTITUENT (TRAITS)	SAMPLE NUMBER	MEAN	SD	EST. MIN	EST. MAX	RSQ
%NDF	101	80.2287	5.078	64.9947	95.4627	0.9712
%ADF	101	67.5621	4.7505	53.3106	81.8136	0.9506

After that, the validation samples were used to validate the developed models and the results (Appendix 3 and Appendix 4) showed that RSQ of %NDF and %ADF models were 0.9213 and 0.9166 which were very close to '1' (Figure 3). As a result, both prediction models are of high quality which means that the values of these traits can be predicted in high accuracy. The result also showed that the S.E.P. values for both %NDF and %ADF were lower than 3*S.E.L. values which also represent good models (Table 2).

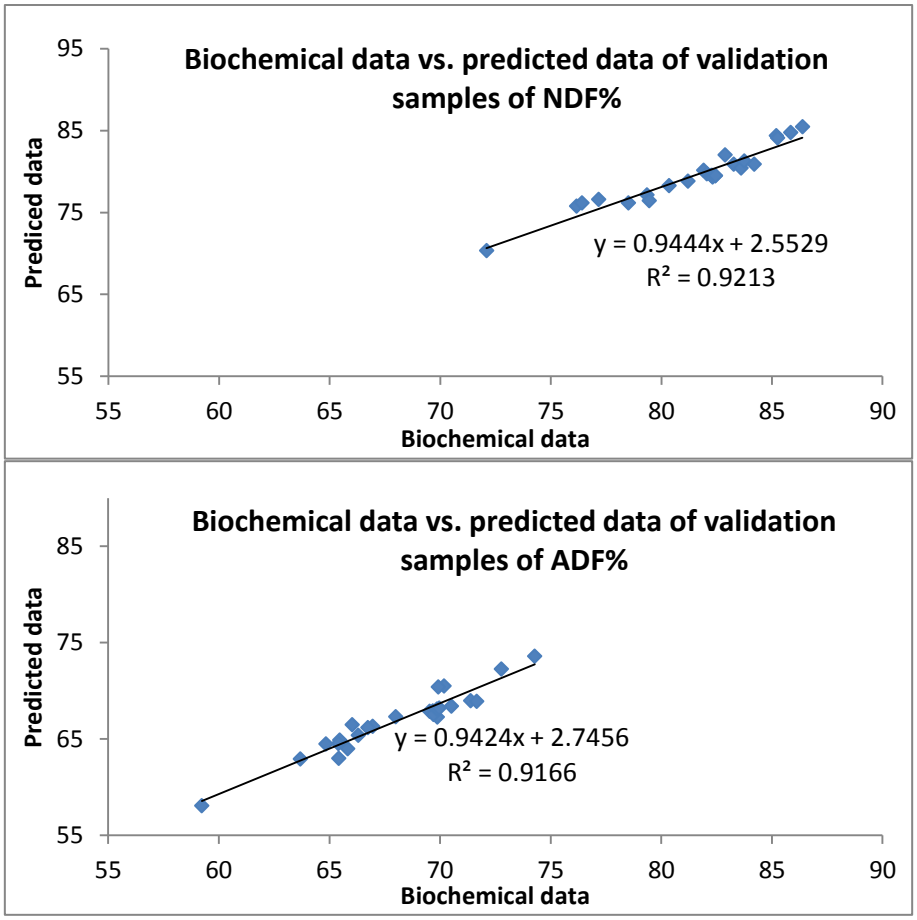

Figure 3 Biochemical data vs. predicted data of validation samples

Table 2 Compare between S.E.P. and S.E.L. to check models

CONSTITUENT (TRAITS)	S.E.P. (S.D. PREDICTED DATA)	S.E.L. (S.D. BIOCHEMICAL DATA)	3*S.E.L.
%NDF	2.185	3.399253871	10.19776161
%ADF	1.503	3.290045549	9.870136646

After the models were developed and validated, the percentage of NDF and percentage of ADF of the samples that were not analysed biochemically were predicted by using the two developed models. Using the predicted data, the samples were sorted by the %NDF and %ADF, and then, ten samples with lowest predicted %NDF, %ADF and ten samples with highest predicted %NDF, %ADF were selected. The ten samples with lowest predicted %NDF and %ADF values were grown in France (Sample No. have '_FNPC' at the end) and the top ten samples with highest predicted %NDF and %ADF values were grown in the Netherlands (Sample No. have '_VDS' at the end) (Table 3). Most samples with lowest %NDF values also had the lowest %ADF values and the samples with highest predicted values also had the same situation. The predicted %NDF and %ADF values of all the 1034 samples show that the samples with the lowest %NDF and %ADF were all cultivated in France and the highest values came from the Dutch location. The plant cultivated in Italy show similar results as the plants cultivated in France. In addition, the predicted values also show that accessions grown in different locations, had significant differences of %NDF and %ADF. For example, the accession 'MH-VDS-304_B3' also known as 'Ivory' shows the lowest %ADF dry matter when grown in France but the

highest %ADF when grown in the Netherlands. This fact may be explained by the effects of different temperatures and light density among the three locations on biomass quality.

The trial locations have different environment conditions; the Netherlands appears to be cooler and has lower light intensity compare with Italy and France. Moreover, it is known that hemp is sensitive to the temperature and light intensity (Pahkala et al., 2008). Therefore, the flowering time will delay when the plant are grown in the Netherlands with cooler temperatures and lower light intensity conditions. As a result, the stem growth of hemp will decrease after flowering (Van der Werf et al., 1994a), and the quality of the cellulose was relatively stable during growth, but lignification may developed rapidly after flowering (Struik et al., 2000). The harvesting time for hemp is also set according to the flowering time which is the time that the maximum yield of fibre is reached (Amaducci et al., 2002; Mediavilla et al., 2001). Because of the delay of the flowering time and a prolonged vegetative growth period, hemp seems to produce higher yield of fibre (Van der Werf et al., 1994a). This finding could be applied to improve the production of hemp fibre and hemp flower related products. On the one hand, to reach higher quantity and quality of fibre, hemp fibre accessions should be cultivated in cooler and lower light intensity conditions like the ones from the North of Europe (i.e. the Netherlands). On the other hand, to improve the production of flowers and seeds, hemp should be cultivated in the South of Europe where the temperatures are warmer and more light intensity is available for the plants.

Table 3 Top ten samples with lowest and highest predicted %NDF and %ADF

	Sample No.	Predicted %NDFdm	Sample No.	Predicted %ADFdm
Lowest	**MH-CRA-401bis_B2_FNPC**	63.231	**MH-CRA-401bis_B2_FNPC**	51.781
	MH-WU-130_B1_FNPC	66.442	**MH-WU-130_B1_FNPC**	54.872
	MH-WU-117_B1_FNPC	66.472	**MH-VDS-304_B3_FNPC**	54.979
	MH-WU-131bis_B3_FNPC	66.981	**MH-WU-117_B1_FNPC**	55.057
	MH-CRA-418_B3_FNPC	67.132	**MH-WU-110_B1_FNPC**	55.626
	MH-WU-107_B2_FNPC	67.617	**MH-WU-107_B2_FNPC**	55.642
	MH-WU-132_B1_FNPC	67.78	**MH-WU-131bis_B3_FNPC**	55.981
	MH-WU-110_B1_FNPC	67.837	**MH-WU-132_B1_FNPC**	56.165
	MH-WU-107_B3_FNPC	68.064	**MH-CRA-418_B3_FNPC**	56.316
	MH-FNPC-223_B2_FNPC	68.074	**MH-CAAS-602_B2_FNPC**	56.365
Highest	**MH-IWNRZ-901_B3_VDS**	87.183	**MH-FNPC-226_B3_VDS**	74.447
	MH-VDS-304_B3_VDS	87.314	**MH-VDS-303_B3_VDS**	74.464
	MH-WU-109_B3_VDS	87.315	**MH-VDS-304_B3_VDS**	74.574
	MH-VDS-303_B3_VDS	87.333	**MH-IWNRZ-902_B2_VDS**	74.605
	MH-FNPC-207_B3_VDS	87.608	**MH-FNPC-201_B3_VDS**	74.606
	MH-LARC-501_B1_VDS	87.766	**MH-WU-119_B3_VDS**	74.714
	MH-FNPC-228_B3_VDS	87.77	**MH-FNPC-228_B3_VDS**	74.935
	MH-FNPC-202_B3_VDS	88.124	**MH-WU-109_B3_VDS**	75.196
	MH-WU-119_B3_VDS	88.423	**MH-FNPC-204_B3_VDS**	75.349
	MH-FNPC-204_B3_VDS	88.701	**MH-IWNRZ-902_B1_VDS**	75.529

Furthermore, on the one hand, for each hemp accession, the SD and the RSD of the same block coming from different locations were calculated (by plot). On the other hand, for each hemp accession, the SD and the RSD were calculated again but using three blocks coming from the same location (by location). The RSD of the predicted %NDF and %ADF within the same accession in the three locations is much higher than the RSD of the three replicates (blocks) in the same location. This result means that the differences among locations are higher than the differences among blocks. Therefore, the differences of these two kinds of RSD give more evidences to the fact that the environment influences the percentage of NDF and ADF.

Finally, the purpose for this project is to determine the cell wall composition which is an important factor to define the hemp fibre quality. Until now, the NDF and ADF treatments have been assessed. Therefore, it is only possible to calculate the percentage of the sum of hemi-cellulose, cellulose, insoluble pectin and lignin of the fibre, the percentage of lignin and cellulose together, and the percentage of hemi-cellulose and insoluble pectin of the fibre. However, to calculate the percentage of the separate components further experiments should be performed such as to assess the percentage of ADL (acid detergent lignin). After the percentage of the separate cell wall components determined, it will be able to find out which hemp accessions have high fibre quality like high cellulose content and low degree of lignification or to find out which growing conditions can produce higher hemp fibre quality.

4 Conclusions

In this minor thesis project, 1034 hemp samples from different origins and cultivated in three different locations were scanned by NIRS, based on the NIRS spectrums of these 1034 samples, 101 samples were selected to do the biochemical analysis to determine the percentage of cell wall composition. After that, using the biochemical and the NIRS spectrum data, two models to predict %NDF and %ADF were developed. Twenty-five samples were selected randomly and used to validate the models. The results show that it is possible to develop high quality prediction models to predict the cell wall composition of hemp. The predicted biochemical data shows that the plants that were grown in France have the lowest %NDF and %ADF, the plant that were grown in the Netherlands have the highest %NDF and %ADF and the percentage of NDF and percentage of ADF in hemp samples are strongly influenced by the environment.

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Appendices

Appendix 1 Biochemical data: percentage of NDF and percentage of ADF from 101 selected samples to develop the prediction models

Position	NIRS code	Average %NDF	Average %ADF
1	**MH-WU-111_B1_CRA**	82.34294946	70.77653086
2	**MH-LARC-501_B1_CRA**	76.52317668	63.52069754
3	**MH-FNPC-236_B1_CRA**	80.98061345	69.03921642
4	**MH-FNPC-216_B1_CRA**	78.68538764	66.29930872
5	**MH-WU-115_B1_CRA**	68.83167036	56.25661152
6	**MH-CRA-420_B1_CRA**	80.573154	69.57230835
7	**MH-VDS-301_B1_CRA**	77.40062989	65.37408866
8	**MH-CAAS-601_B1_CRA**	78.65678968	63.99042663
9	**MH-CRA-408bis_B1_CRA**	78.47170145	65.81058638
10	**MH-VDS-303_B1_CRA**	76.0902624	64.0102752
11	**MH-FNPC-238_B2_FNPC**	78.07464077	65.78532112
12	**MH-WU-116_B2_FNPC**	74.70004501	61.27398686
13	**MH-FNPC-225_B2_FNPC**	70.99581641	59.80658229
14	**MH-WU-128_B2_FNPC**	79.43476594	64.75812224
15	**MH-FNPC-230_B2_FNPC**	81.68532998	69.64612469
16	**MH-VDS-304_B2_FNPC**	71.05173817	60.29736924
17	**MH-FNPC-214_B2_FNPC**	76.35353446	64.8976713
18	**MH-WU-123_B2_FNPC**	72.21527143	59.30007245
19	**MH-WU-117_B2_FNPC**	69.5576361	57.50059166
20	**MH-WU-126_B2_CRA**	78.61886994	67.79019776
21	**MH-FNPC-243_B2_CRA**	77.3718512	66.76774147
22	**MH-FNPC-233_B2_CRA**	76.24564356	66.09517361
23	**MH-LARC-501_B3_CRA**	79.02775179	66.2574688
24	**MH-FNPC-224_B2_FNPC**	77.0257974	66.91471878
25	**MH-CAAS-603_B2_FNPC**	70.62220429	57.3051835
26	**MH-FNPC-210_B1_FNPC**	76.9924186	64.41804399
27	**MH-FNPC-254_B1_FNPC**	76.9555793	64.65745612
28	**MH-WU-123_B1_FNPC**	82.49020502	67.23578473
29	**MH-WU-118_B1_FNPC**	72.10856362	58.987102
30	**MH-FNPC-237_B1_FNPC**	78.91765695	66.70178688
31	**MH-FNPC-246_B3_CRA**	79.07342906	66.2696592
32	**MH-CAAS-604_B3_CRA**	78.49434061	64.79224921
33	**MH-IWNRZ-902_B3_CRA**	81.43304002	71.1328314
34	**MH-WU-115_B3_CRA**	78.45048792	65.21268433
35	**MH-CRA-417bis_B3_CRA**	79.77957223	66.51571477
36	**MH-WU-125_B1_FNPC**	71.59750369	59.21505408
37	**MH-CRA-419_B1_FNPC**	72.74103404	61.56244192
38	**MH-CAAS-603_B1_FNPC**	69.45667832	56.25767519

39	**MH-WU-116_B3_FNPC**	75.33829244	65.12882008
40	**MH-FNPC-254_B3_FNPC**	69.66366151	58.49438951
41	**MH-WU-105_B3_FNPC**	70.42749703	60.29806713
42	**MH-WU-115_B3_FNPC**	71.76608991	57.36975194
43	**MH-FNPC-211_B3_FNPC**	71.91370625	61.64992937
44	**MH-FNPC-208_B3_FNPC**	77.16950381	67.22698767
45	**MH-WU-102_B3_FNPC**	74.49738251	63.49579055
46	**MH-CRA-408bis_B3_CRA**	72.36681683	61.97534818
47	**MH-WU-110_B3_CRA**	73.51415762	60.43379482
48	**MH-CRA-411bis_B3_VDS**	85.22713454	70.91367715
49	**MH-FNPC-234_B3_VDS**	86.03755092	72.27087056
50	**MH-WU-123_B3_VDS**	86.12664732	72.63001807
51	**MH-WU-127_B3_VDS**	83.64627159	70.91861251
52	**MH-CAAS-601_B3_VDS**	82.19058296	65.42443083
53	**MH-FNPC-248_B3_VDS**	86.54923536	72.63276353
54	**MH-WU-104_B3_VDS**	83.51529185	70.84393987
55	**MH-FNPC-254_B3_VDS**	85.06451312	70.45960335
56	**MH-FNPC-210_B3_VDS**	84.33776525	71.97503316
57	**MH-FNPC-209_B3_VDS**	85.36724422	72.9669059
58	**MH-WU-131bis_B3_VDS**	84.24921067	67.49753212
59	**MH-WU-118_B3_VDS**	85.95929743	71.38323431
60	**MH-IWNRZ-901_B3_VDS**	86.40758531	73.95922309
61	**MH-IWNRZ-903_B3_VDS**	85.48503777	72.20091013
62	**MH-CRA-412_B3_VDS**	84.80603976	70.71220091
63	**MH-CAAS-603_B3_VDS**	81.70096462	66.90645629
64	**MH-CRA-409_B3_VDS**	83.49843111	68.92316485
65	**MH-CRA-405bis_B3_VDS**	83.55849523	71.84922464
66	**MH-FNPC-208_B3_VDS**	86.1841058	73.58695706
67	**MH-CRA-414_B3_VDS**	84.20000969	71.66119622
68	**MH-WU-119_B3_VDS**	88.15232644	74.68209126
69	**MH-FNPC-244_B3_VDS**	82.70880287	70.03911738
70	**MH-FNPC-252_B3_VDS**	82.92014844	67.98956332
71	**MH-WU-107_B3_VDS**	85.61281268	72.2924009
72	**MH-WU-106_B3_VDS**	87.91528515	73.99679109
73	**MH-WU-115_B3_VDS**	84.57259492	70.76801044
74	**MH-WU-131bis_B1_VDS**	82.62152164	68.46118843
75	**MH-FNPC-219_B1_VDS**	83.97695938	73.50363241
76	**MH-FNPC-231_B1_VDS**	83.32916825	71.77608108
77	**MH-FNPC-204_B1_VDS**	84.19591436	74.21867973
78	**MH-FNPC-202_B1_VDS**	82.7287538	71.67660377
79	**MH-LARC-501_B1_VDS**	87.04569243	72.26780757
80	**MH-UOY-801_B1_VDS**	84.89563921	70.68048002
81	**MH-FNPC-228_B1_VDS**	85.02002477	73.25787933
82	**MH-WU-110_B1_VDS**	84.37594202	70.3829188

83	**MH-CRA-402bis_B1_VDS**	78.34893344	65.84092601
84	**MH-WU-115_B1_VDS**	85.25481269	70.78289631
85	**MH-WU-111_B1_VDS**	85.54792484	72.14342879
86	**MH-FNPC-226_B1_VDS**	80.62813303	68.95072036
87	**MH-AGM-703_B1_VDS**	86.60153339	73.38772516
88	**MH-CAAS-604_B1_VDS**	80.5824464	68.74750045
89	**MH-FNPC-214_B2_VDS**	83.58660884	70.37097439
90	**MH-CRA-402bis_B2_VDS**	80.81722955	66.16871624
91	**MH-WU-106_B2_VDS**	83.74537997	71.62944474
92	**MH-CRA-406_B2_VDS**	81.88023654	66.61586851
93	**MH-CRA-420_B2_VDS**	84.57120766	73.54007347
94	**MH-WU-104_B2_VDS**	82.99927384	70.66981824
95	**MH-WU-128_B2_VDS**	84.07679088	69.24134795
96	**MH-FNPC-217_B2_VDS**	83.04389433	70.94768669
97	**MH-FNPC-209_B2_VDS**	82.78378337	71.33133611
98	**MH-CAAS-603_B2_VDS**	78.40646615	65.58499409
99	**MH-WU-113_B2_VDS**	81.55767611	70.1524244
100	**MH-VDS-302_B2_VDS**	82.99751729	70.53182016
101	**MH-IWNRZ-902_B2_VDS**	84.8011406	73.3484618

Appendix 2 Biochemical data: percentage of NDF and percentage of ADF from 25 selected samples to validate the prediction models

NIRS code	Average %NDF	Average %ADF
MH-CRA-413bis_B2_FNPC	72.08889021	59.23209371
MH-LARC-501_B2_FNPC	76.15780181	63.6787083
MH-FNPC-244_B1_FNPC	76.40821734	65.46396779
MH-FNPC-218_B3_FNPC	77.16704959	65.40478154
MH-WU-129_B3_CRA	78.51261605	65.82107236
MH-CRA-401bis_B3_CRA	79.34682433	65.42431792
MH-WU-127_B1_CRA	79.44613595	64.83541061
MH-CRA-407_B2_CRA	80.3439828	66.30715335
MH-CRA-416_B2_CRA	81.20278495	66.95209176
MH-CAAS-602_B2_VDS	81.91246723	66.0259911
MH-FNPC-228_B1_CRA	82.06009742	69.6746951
MH-WU-101_B3_CRA	82.25141663	69.8784481
MH-IWNRZ-903_B1_CRA	82.29986893	67.98845531
MH-AGM-704_B1_CRA	82.31277917	66.74315342
MH-WU-104_B1_CRA	82.43170115	69.52531813
MH-FNPC-219_B2_CRA	82.43882569	69.70041024
MH-UOY-801_B3_FNPC	82.88587436	69.86222484
MH-FNPC-230_B3_CRA	83.26563576	70.51548182
MH-WU-119_B2_CRA	83.60064696	69.96808976
MH-FNPC-239_B3_CRA	83.74444473	71.37949799
MH-FNPC-231_B2_CRA	84.1908274	71.66123292
MH-AGM-702_B1_VDS	85.19983106	70.17457187
MH-WU-121_B2_VDS	85.25559119	69.92473437
MH-FNPC-241_B1_VDS	85.8444996	72.77136594
MH-FNPC-212_B3_VDS	86.38191426	74.27649395

Appendix 3 Biochemical data and predicted data of percentage of NDF of validation samples

Biochemical data and predicted data of %NDF of validation samples							
SEP:		2.185			Num. Samps:	25	
Means:		81.47	79.496	Std. Devs:	3.399	3.345	
Bias:		1.974*			Bias Limit:	0.584	
SEP(C):		0.957			SED(C) Limit:	1.265	
Slope:		0.976			RSQ:		0.921
Ave. Global H:	0.706			Ave. Neigh. H:	0.147		
Pos.	**Sample No.**	**Lab**	**ANL**	**Residual**	**Bias**	**GH1**	**NH1**
1	**MH-CRA-413bis_B2_FNPC**	72.089	70.35	1.739	-0.235	0.507	0.072
2	**MH-LARC-501_B2_FNPC**	76.158	75.788	0.37	-1.604	0.456	0.229
3	**MH-FNPC-244_B1_FNPC**	76.408	76.163	0.245	-1.729	0.742	0.117
4	**MH-FNPC-218_B3_FNPC**	77.167	76.597	0.57	-1.404	0.684	0.094
5	**MH-WU-129_B3_CRA**	78.513	76.169	2.344	0.37	0.59	0.212
6	**MH-CRA-401bis_B3_CRA**	79.347	77.136	2.211	0.237	0.687	0.165
7	**MH-WU-127_B1_CRA**	79.446	76.453	2.993*	1.019	0.713	0.118
8	**MH-CRA-407_B2_CRA**	80.344	78.297	2.047	0.073	0.474	0.103
9	**MH-CRA-416_B2_CRA**	81.203	78.841	2.362	0.388	0.788	0.148
10	**MH-CAAS-602_B2_VDS**	81.912	80.158	1.755	-0.219	1.065	0.176
11	**MH-FNPC-228_B1_CRA**	82.06	79.734	2.326	0.352	0.747	0.137
12	**MH-WU-101_B3_CRA**	82.251	79.553	2.698*	0.724	0.6	0.13
13	**MH-IWNRZ-903_B1_CRA**	82.3	79.538	2.762*	0.788	0.677	0.156
14	**MH-AGM-704_B1_CRA**	82.313	79.329	2.984*	1.01	0.512	0.068
15	**MH-WU-104_B1_CRA**	82.432	79.507	2.925*	0.951	1.197	0.117
16	**MH-FNPC-219_B2_CRA**	82.439	79.486	2.953*	0.979	0.791	0.109
17	**MH-UOY-801_B3_FNPC**	82.886	82.046	0.84	-1.134	1.123	0.192
18	**MH-FNPC-230_B3_CRA**	83.266	80.909	2.357	0.383	0.601	0.153
19	**MH-WU-119_B2_CRA**	83.601	80.423	3.178*	1.204	0.628	0.13
20	**MH-FNPC-239_B3_CRA**	83.744	81.28	2.465	0.491	0.806	0.127
21	**MH-FNPC-231_B2_CRA**	84.191	80.908	3.283*	1.309	1.124	0.136
22	**MH-AGM-702_B1_VDS**	85.2	84.39	0.81	-1.164	0.67	0.275
23	**MH-WU-121_B2_VDS**	85.256	84.108	1.148	-0.826	0.298	0.191
24	**MH-FNPC-241_B1_VDS**	85.844	84.777	1.067	-0.907	0.819	0.166
25	**MH-FNPC-212_B3_VDS**	86.382	85.466	0.916	-1.058	0.356	0.162

Appendix 4 Biochemical data and predicted data of percentage of ADF of validation samples

Biochemical data and predicted data of %ADF of validation samples							
SEP:		1.503			Num. Samps:	25	
Means:		68.128	66.951	Std. Devs:	3.29	3.239	
Bias:		1.177*			Bias Limit:	0.6	
SEP(C):		0.954			SED(C) Limit:	1.3	
Slope:		0.973			RSQ:		0.917
Ave. Global H:	0.706			Ave. Neigh. H:	0.147		
Pos.	**Sample No.**	**Lab**	**ANL**	**Residual**	**Bias**	**GH1**	**NH1**
1	**MH-CRA-413bis_B2_FNPC**	59.232	58.095	1.138	-0.039	0.507	0.072
2	**MH-LARC-501_B2_FNPC**	63.679	62.928	0.75	-0.426	0.456	0.229
3	**MH-FNPC-244_B1_FNPC**	65.464	64.886	0.578	-0.599	0.742	0.117
4	**MH-FNPC-218_B3_FNPC**	65.405	64.516	0.888	-0.288	0.684	0.094
5	**MH-WU-129_B3_CRA**	65.821	63.991	1.83	0.653	0.59	0.212
6	**MH-CRA-401bis_B3_CRA**	65.424	62.998	2.427	1.25	0.687	0.165
7	**MH-WU-127_B1_CRA**	64.835	64.499	0.337	-0.84	0.713	0.118
8	**MH-CRA-407_B2_CRA**	66.307	65.41	0.897	-0.279	0.474	0.103
9	**MH-CRA-416_B2_CRA**	66.952	66.324	0.628	-0.548	0.788	0.148
10	**MH-CAAS-602_B2_VDS**	66.026	66.491	-0.465	-1.641	1.065	0.176
11	**MH-FNPC-228_B1_CRA**	69.675	67.919	1.756	0.58	0.747	0.137
12	**MH-WU-101_B3_CRA**	69.878	67.289	2.590*	1.413	0.6	0.13
13	**MH-IWNRZ-903_B1_CRA**	67.988	67.33	0.658	-0.518	0.677	0.156
14	**MH-AGM-704_B1_CRA**	66.743	66.195	0.548	-0.629	0.512	0.068
15	**MH-WU-104_B1_CRA**	69.525	67.88	1.645	0.469	1.197	0.117
16	**MH-FNPC-219_B2_CRA**	69.7	67.557	2.143	0.967	0.791	0.109
17	**MH-UOY-801_B3_FNPC**	69.862	68.104	1.758	0.582	1.123	0.192
18	**MH-FNPC-230_B3_CRA**	70.515	68.419	2.096	0.92	0.601	0.153
19	**MH-WU-119_B2_CRA**	69.968	68.227	1.741	0.564	0.628	0.13
20	**MH-FNPC-239_B3_CRA**	71.38	68.988	2.392	1.215	0.806	0.127
21	**MH-FNPC-231_B2_CRA**	71.661	68.908	2.754*	1.577	1.124	0.136
22	**MH-AGM-702_B1_VDS**	70.175	70.523	-0.348	-1.525	0.67	0.275
23	**MH-WU-121_B2_VDS**	69.925	70.421	-0.496	-1.672	0.298	0.191
24	**MH-FNPC-241_B1_VDS**	72.771	72.269	0.503	-0.674	0.819	0.166
25	**MH-FNPC-212_B3_VDS**	74.276	73.611	0.665	-0.511	0.356	0.162