Native stinging nettle (*Urtica dioica L.*) growing spontaneously under short rotation coppice for phytomanagement of trace element contaminated soils: fibre yield, processability and quality

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Abstract

This work assesses the potential of stinging nettle (*Urtica dioica* L.) growing on trace element contaminated soils to produce fibres for material applications. The nettles studied in this work grew spontaneously and dominated the vegetation cover in poplar short rotation coppices planted for the phytomanagement of lands contaminated by trace elements. Two sites were studied, contaminated by Hg for the first one and a mix of As, Cd, Pb and Zn for the second one. Results show that, for the considered soils, the contaminant contents in nettle bast fibres were at low levels, comparable to those collected at unpolluted control areas. It makes it possible to consider this biomass for material use. The measured matter yield was lower than those obtained with traditional fibre crops cultivated in Europe on agricultural lands. However, the tensile properties of the bast fibres mechanically extracted without field retting or prior alkaline treatment were equal to or better than those of industrial hemp and flax, making spontaneous nettles an interesting supplement to traditional European fibre crops for material applications.

Key words: Nettle fibre, phytomanagement, contaminated soils, fibre yield, tensile properties

1 Introduction

Environmental contamination is a threat to global sustainable development. The United Nations (UN) Sustainable Development Goals (SDGs) have a strong focus on reducing and managing environmental pollution. Among the various soil phytoremediation options, phytostabilisation is a sustainable and profitable use of marginalized lands (Pilon-Smits, 2005) that can remediate soil layers in contact with the roots and at the same time provide tree biomass (Robinson et al., 2006), which can be used to reach the targets for the use of renewable energy sources (Ciadamidaro et al., 2017; Mench et al., 2010). It creates value from contaminated land while minimising environmental risks and even rendering ecosystemic services by promoting biodiversity, improving the carbon storage and limiting soil erosion. Interestingly, it has been observed simultaneous growth of poplars under short rotation coppice (SRC) cropping systems and spontaneous dioecious nettle in phytomanaged land sites, thus constituting a multilevel canopy. Biomass from nettle could be used to extend the productivity of the marginal land from SRC alone, whilst still maintaining a functioning phytomanagement system of land rehabilitation. This nettle biomass could constitute a relevant fibre source and thus contribute to answer the increasing demand in plant fibres for material application, by mitigating at the same time the land-use conflict between the needs of food and non-food production. Indeed, recent literature underlines that the use of marginal land is a sustainable method to expand purpose-grown biomass and essential for meeting the emerging massive requirement for biomass in the future (Mohanty et al., 2018).

Urtica dioica L., often called common nettle or stinging nettle, is a herbaceous perennial flowering plant in the family *Urticaceae*, living up to 10 to 15 years and growing up to 2 m in height (Gravis, 1885). Each stem is formed of a succession of nodes and internodes containing bast fibres in the bark. At each node, two opposite leaves and four cauline stipules are inserted. As for all the fibre crops, the fibre features depend on the living conditions, the plant maturity, the position in the stem (Gravis, 1885). The morphological features of nettle bast fibres were accurately characterised in historical and encyclopaedic works (Gravis, 1885; Vétillart, 1876) and more recently in the scientific literature (Di Virgilio, 2013). The fibres, in limited amount, are distributed in small numbers in the parenchyma in an irregular manner. Most of the fibres are oval in shape, with a diameter generally comprised between 20 and 40 µm. They have a relatively thin wall and thus a very large inner cavity. The fibre wall is mainly composed of cellulose, hemicellulose and lignin, in amount generally comprised in the following ranges 53-86, 4-12.5 and 2-10% respectively, depending on the plant age, maturity, growing and processing conditions (Bacci et al., 2010; Di Virgilio, 2013; Di Virgilio et al., 2015). When nettle is cultivated, dry stalk yields could be comprised between 3 and 12 $t.ha^{-1}$ (Bacci et al., 2009; Dreyer et al., 1996; Vogl and Hartl, 2003). As the two other European fibre crops (flax and hemp), nettle (*Urtica dioica* L) has a long history as a textile fibre and its potential as reinforcement fibres in composite applications was also investigated more recently (Bacci et al., 2010; Di Virgilio et al., 2015; Dreyer et al., 2002; Franck, 2005; Harwood and Edom, 2012; Vogl and Hartl, 2003). Indeed, its bast fibres have remarkable tensile strength and fineness, along with a low density. Average tensile strength and rigidity up to 1600 MPa and 90 GPa have been reported in literature (Bodros and Baley, 2008; Davies and Bruce, 1998; Lanzilao et al., 2016). The stalks and shives can also be used to manufacture fibreboards (Akgül, 2013). As underlined by Di Virgilio et al. (Di Virgilio et al., 2015), despite a high market potential, the products made from nettle are currently more a result of curiosity rather large-scale industrial production, mostly due to lack in crop and post-harvest management.

The present study is part of a wider research project, which main objective is the multi-purpose valorisation of wood and plant fibres produced on marginal lands, and focused more precisely on the phytomanagement of trace element (TE) contaminated lands. It aims at investigating the potentialities of nettle fibres growing spontaneously under SRC grown poplar at two phytomanagement sites, for material application. Hg is the main contaminant at the first site due to the chlor-alkali process (Durand et al., 2017), whereas As, Cd, Pb and Zn are the main contaminants at the second site due to contamination of deposit sediments from the canal Escaut. (Ciadamidaro et al., 2017). This work addresses several issues such as the potential accumulation of TE in the bast fibres. The potential influence of contamination on the plant and fibre yields and on the physical properties of the bast fibres are also studied by comparing the data obtained on the contaminated sites to data from literature collected on non-contaminated soils. Regarding TE accumulation in plants, previous studies (revealed that nettle is a poor TE accumulating specie when grown on contaminated soils, as compared to woody accumulating or hyperaccumulating species (Phanthavongsa et al., 2017). Indeed, in our previous work, Zn and Cu contents in nettle leaves collected at a sediment landfill were within physiological ranges (<100 mg/kg DWt for Zn and <10 mg/kg DWt for Cu), and Cd content was < 10 mg/kg DWt) (Phanthavongsa et al., 2017); (Codling and Rutto, 2014; Tack and Verloo, 1996). However, there is no clear conclusion regarding the content in stems and bast fibres. For other fibre crops, such hemp, it was previously demonstrated that bast fibres and shives were not affected by TE contamination (Linger et al., 2002). The fibre features (fineness, strength) were also not altered by the contamination making this type of crop a relevant candidate for phytostabilisation option.

2 Materials and methods

2.1 Site description

The experimental sites are located in Tavaux (France, Bourgogne Franche Comté region, lat. 47° 5' 5.98'' N. 5° 19' 44.0322" E) and Fresnes-sur-Escaut (France, Hauts-de-France region, lat. 50° 25' 4'' N, 3° 35' '' E). Briefly, the Tavaux site (S1)(Fig.2-S1) investigated in the present study was exploited from the 1950s to 2003 as a storage area for sediments originated from effluents produced during the electrolytic processes associated with a Hg cell chlor-alkali activity. The Fresnes-sur-Escaut (S2)(Fig.2-S2) site was a deposit site of sediments from the canal Escaut. Due to transport of metal ores in the past, sediments were contaminated by TE such as Cd, Zn, As and Pb. Soils at both sites differed in their composition. The full description of the Tavaux and Fresnes-sur-Escaut sites and soils are provided in (Durand et al., 2017) and (Ciadamidaro et al., 2017), respectively. The main meteorological parameters during the growth period at the two sites are summarized in table 1. At both sites, nettle grew spontaneously under the woody cover (Fig. 1).

Two other uncontaminated site (control) were used as reference to compare contamination values. The control site in France is a natural area located at Courcelle-les-Montbéliard, 140 km east of the site S1, not directly influenced by the point-source. (Yung et al., 2019) gives a description of this site. The other control site is located in Belgium at Ghent region at 120 km north-east of the site S2. A description of this site is given in (Tack and Verloo, 1996).

2.2 Experimental design

Within the BIOFILTREE project (Ciadamidaro et al., 2017), four type of field plots were set-up in March (S2) and April (S1) 2011. The four type of plots consisted in i) poplar (Skado clone) monocultures, ii) poplar (I214 clone) monocultures, iii) Skado-alder associations, iv) I214-alder associations, with or without inoculation. Only the poplar monocultures have been considered in the present study. On the two sites, non rooted poplar cuttings (1.5 m length) of the clones Skado (*P. trichocarpa x P. maximowiczii* section Tacahamaca) and I214 (*P. deltoides x P. nigra* section Aigeiros) were used, with a final tree density of 2.200 plants.ha^{-1}, each plot therefore having a surface of 28 x 18 m for S1 and 36 x 12 m for S2. Both clones were selected for their growth yield and low leaf TE accumulation capacities, from the previous clonal field trial PHYTOPOP (Pottier et al., 2015). Figure 2 represents the implantation of the two described sites. Full description of the sites is provided in (Phanthavongsa et al., 2017).

2.3 Nettle harvesting

During the first year (Y1), half of each plot area (approximately 250 m^2) was harvested for three selected plots (T.I1, T.I2 and T.I3) on S1 (Fig. 2, Fig.3a, b and c) on the 28th of September 2017. The second year (Y2), an area of 50 m^2 was harvested in the plot TS of S1 on the 8th of June 2018, and an area of 156 m^2 in the plots FS, P4, P5 and P30 on S2 on the 18th of July 2018. The harvesting strategy was mainly driven by observations of the nettle development as well as the competing species. At each harvest, stems were cut at soil level using scythes and shears. The aerial parts of the cut plants were then immediately picked up by hand and baled, without prior field retting. The stems were kept aligned in the bundles (Fig. 3d). The bundles were picked up the same day and stored in indoor spaces for a minimal period of 3 months.

2.4 Fresh and dry matter yields

The nettle bales were weighed in the field just after harvesting to determine the mass of fresh plant materials (M_{fresh}), and then weighed again after air-drying (at least 4 months of conditioning in indoor spaces) to determine the mass of dried plant materials (M_{dry}). The leaves were then manually picked off. The remaining stems were further weighed to determine the mass of dried stems (M_{stem}). The yields were then estimated by calculating the ratio between the masses and the plot areas. The yields in fresh matter, dry matter and dry stems are noted η_{fresh}, η_{dry}, η_{stem}, respectively.

2.5 Stem length and diameter

Stems were analysed in terms of dimensions by selecting randomly 30 stems in each harvested batch. Stem diameters were measured for each stem in three positions along the stem height (top, middle and base parts of the stem) using a calliper with a precision to 0.01 mm. For each position, two measurements were done according to the two main directions of the transversal cross-section area. The average diameter for this position was then calculated from these two measurements. The mean diameters measured in these respective positions are noted Dtop, Dmid, Dlow. The length (L) was measured using a graduated steel rule with a precision of 0.1 cm.

2.6 Stalks processing and fibre extraction.

The stems of the Y2-S1 batch were mechanical processed using a Laroche (France) Cadette 1000 “all fibre” extraction device. This machine is a three-drum opener with a 1000 mm wide feed belt (Fig. 4a). At the entrance of each of the three modules, the feeding of the fibrous material was provided by a pair of rolls, one smooth and the other grooved (made of rubber) (Fig. 4b). Then, each module was equipped with a cylinder with nails, *i.e.* the extracting roller (or fibre extraction roller) (Fig. 4c). Its distance from the metal trough located at the module inlet is adjustable (distance A in Fig. 4b), as well as its rotational speed (from 750 to 1800 rpm). Under the extracting roller, a trap door allowed the evacuation of shives by gravity. The trap door opening is also adjustable and, for this study, it was set at its maximum to favour the shives removal. At the end of the module, there was also a perforated cylinder at which ventilation was applied. The latter had three distinct functions: (i) the de-dusting of the material, (ii) the formation of the fibre lap, and (iii) its transfer to the next module or the outlet. Each de-dusting fan was equipped with a motor having a 2865 rpm maximum rotation speed.

During the experiments, the operating parameters used were inspired by two previous studies dealing with the mechanical extraction of technical fibres from oleaginous flax (Ouagne et al., 2017) and hemp (Grégoire et al., 2019) straws, respectively. From modules 1 to 3, distance A were 1.9 mm, 1.4 mm and 1.1 mm, respectively. The inlet flow rates of nettle stems were approximately 125 kg/h, corresponding to a 2.5 m/min feed belt speed. The transmission speed of the lap from module 1 to module 2 was 2.2 m/min, and it was 2.1 m/min from module 2 to module 3. Finally, a 1.7 m/min speed was used for the output belt. In each of the three modules, a 750 rpm rotation speed was chosen for the extracting roller. Looking at the de-dusting fans, the rotation speed of the motor was 1500 rpm for module 1, and 2000 rpm for the next two modules.

Since the nettle straws were not field retted, three different conditionings before processing were tested to evaluate their influence on the nettle fibre yield and quality. Prior to these treatments, the straws were stored during 3 months.

- Condition 1 (C1): as-received. The nettle straw was processed as-received after the 3 months of storage. Its average moisture content was approximately 10.4%.
- Condition 2 (C2): moistened. The nettle straw was sprayed with water to re-moisturise the matter (during 15 hours). Its average moisture content was thus approximately 22.0%.
- Condition 3 (C3): soda treatment. The nettle straw was immersed for 2 h and at 60 °C in a soda solution (2%) and then thoroughly rinsed with water until reaching a pH value comprised between 7 and 8. The nettle straw was then oven-dried inside a ventilated oven at 50 °C and during 16 h. The moisture content before mechanical processing was 16.5%.

After the mechanical processing step, the fractions of the resulting matters were determined. These matters were divided into three categories: a fibre lap containing the extracted bast fibres, shives and dust. The content of impurities inside the fibre lap (*i.e.* remaining shives and dust) was also evaluated. Indeed, the lap collected at the outlet of the "all fibre" extraction device consisted not only in bast fibres but also in some remaining shives and dust, both trapped inside the lap. From each of the three generated laps, a 50 g sample was collected to determine the content of impurities inside the lap, and thus the real bast fibre content. A mechanical sieving was performed on the lap sample for 5 min in order to drop shives and dust. Then, the residual shives that remained trapped were collected manually.

Moisture content of the fibrous materials at the inlet of the "all fibre" extraction device and out of the process was determined according to standard ISO 665:2000.

2.7 Fibre diameter

In order to characterize the morphology of the bast fibres and determine their diameters, fresh transverse cross-sections were cut from the stems using razor blades at different locations and different development and maturity stages. Cytochemical staining of lignin was achieved on sections using the Wiesner reagent (phloroglucinol-HCl) that colored lignin into purple-red and assist in the definition of the fibre wall outlines. The observations were made on a microscope Keyence VHX 5000 series with a magnification of 20.

2.8 Single fibre tensile properties

Single fibre tensile tests were performed on nettle fibres isolated by hand from the batch Y2-TS and Y2-FS, and from the mechanically processed batch Y2-TS. The fibres were first glued on a paper frame and then examined using an optical microscope to determine their external width. The average width of each fibre was computed by obtaining ten measurements equally spaced along its length. A Bose Electroforce 3230 machine was used to perform the monotonic tensile tests up to failure. The paper frame supporting each fibre was clamped onto the testing machine and then cut prior to the beginning of each test. The clamping length was 10 mm. Fibres were tested at a constant crosshead displacement rate of 0.01 $mm.s^{-1}$. The applied force was measured with a load sensor of 22 N with a resolution of approximately 10 mN, and the displacement was measured using an LVDT with a resolution of approximately 0.5 µm. The sample elongation and the load were recorded continuously. The strain was calculated as the elongation divided by the initial fibre length, taking into account the compliance of the loading frame.

The effective cross-section was determined using the mean external width, assuming that the fibre was perfectly cylindrical and the lumen area neglected. The stress was calculated using the applied force and the evaluated initial cross-section mean value of the fibre. The variation of the cross-section during the tensile loading was neglected. The tensile strength (σ_R) and the strain at failure (ε_R) were determined, and the apparent tangent modulus of rigidity (E) was computed on a strain range comprised between 0.2% and the strain at failure. During the fibre preparation, and using only optical microscopy, it is sometimes difficult to discriminate single fibres against small bundle of fibres. As a result, some small bundles of nettle fibres were also tested. This can be easily detected on the shape of the resulting stress-strain curve. In a strain-controlled situation, the force drops abruptly when an individual fibre breaks. Thus, a criterion was introduced to identify during the data processing fibre bundles. This one is based on the detection of sudden stress drop (set at 5 MPa) between two successive measure points.

2.9 Determination of TE content

Before analyses, dried nettle stems and leaves from S1 and S2 and non contaminated soil (control) were separated, air-dried and ground into a homogenous powder in a Mixer Mill, for 4 min at 30 Hz and 7 min at 30 Hz for leaves and stems, respectively. We also isolated nettle fibres for Hg analysis. Cd, Cu, Hg, Pb and Zn concentrations were measured in the soil and different parts of nettles. Soil samples were ice-dried and sieved (0.2 mm particle size). The total element concentrations (Al, Cd, Fe, Mn, Pb, S, Zn) in the soil from S1, S2 and control were measured following the acid digestion of 500 mg of sample (DigiPREP system, SCP Sciences, Courtaboeuf, France) using a mix of 2 mL of 67% nitric acid, 6 mL of 34% hydrochloric acid and 2 mL of 48%

hydrofluoric acid (Ciadamidaro et al., 2017). $CaCl_2$-extractable fractions of TE were determined in soil samples after shaking 2.5g of dried soil in 25 ml of a 0.01 M solution of $CaCl_2$ during 3 hr (140 g / min^{-1}) (Houba et al., 1996). The soil mixtures were filtered on filtration units at 0.45 µm (Millipore). Eluates were acidified to a pH < 2 for preservation. TE in plant and soil samples were analysed using inductively coupled plasma atomic emission spectrometry (ICP-AES, Thermo Fischer Scientific, Inc., Pittsburgh, USA). Hg was measured using the atomic absorption spectrophotometry AMA-254 cold vapor atomic absorption (CV-AAS) Hg analyser (Altec Co., Czech Republic), using standard conditions (45 s drying, 150 sheating, 45 s cooling) and the certified reference material (CRM), i.e. Oriental Basma Tobacco leaves (INCT-OBTL-5). All the results are expressed as µg/kg of the dry weight (DW).

2.10 Statistical analysis

The dimensional and mechanical data presented in this paper are the mean values, standard deviations and the parameters of a log-normal distribution law identified by inverse method.

The best distribution function for each property was identified using the EasyFit software by minimising the criterion of distances from the distribution diagram. The distribution functions were selected among the classical ones. The log-normal distribution (*LogN*(μ,λ))(Eq. 1) was found to be more realistic and is preferred to the other distributions. This distribution is a two-parameter family of continuous probability distributions. μ and λ are the expectation and standard deviation of x and ln(x), respectively.

$$LogN(\mu,\lambda):\ f(x)=\frac{1}{x}\frac{1}{\lambda\sqrt{2\pi}}e^{-\frac{1}{2}\left(\frac{\ln(x)-\mu}{\lambda}\right)^2} \qquad (1)$$

3 Results and discussion

3.1 Trace element content of soils and harvested biomasses

The Fresnes-sur-Escaut soil was highly contaminated by Cd, Pb and Zn, and Cu to a lesser extent, which confirms our previous studies (Ciadamidaro et al., 2017; Phanthavongsa et al., 2017). However the nature of the soil rendered these TE poorly available, with $CaCl_2$ extractable fraction represented 0.2, 0.4, 0.02 and 0.04% of the total fraction for Cd, Cu, Pb and Zn, respectively (Table 2). Cu, Zn and Pb contents in nettle collected at the Fresnes-sur-Escaut site ranged within physiological levels (Kabata-Pendias, 2011; Kalra et al., 1998), and did not significantly differed between tissues (Table 2). Cd contents in the various nettle tissues were within the ranges found in plants grown in agricultural soils, and always much lower than Cd concentrations measured in poplar or salix leaves (from 2 to 14 mg/kg DW) collected at the same site (Phanthavongsa et al., 2017). Additionally, the TE concentrations in nettle found in our study were within the ranges of concentrations measured in nettles collected at uncontaminated sites in Belgium near the location of the site S2. (Tack and Verloo, 1996). These TE concentrations were also well below the critical levels set up for plant tissues (Kabata-Pendias, 2011), at which growth depression may be observed, i.e. 10-20 and 100-500 mg/kg DW for Cd and Zn, respectively.

The Tavaux soil was contaminated by Hg, with concentrations around 6 mg/kg DW, as previously found (Durand et al., 2017; Maillard et al., 2016). At the Tavaux site, translocation of Hg to nettle tissues remained low, as Hg contents in leaves did not exceed 0.01 mg/kg DW (Table 2). These concentrations were comparable to those measured at our French control site (mean 0.006 mg/kg DW, Table 2). Hg content of nettle fibres was below the limit of quantification. The Hg concentrations in nettle leaves were far below the threshold set to 0.2 mg/kg for concentrations tolerable in agronomic crops and within the ranges measured in food plants (0.006-0.139 mg/kg DW) in various countries (Kabata-Pendias, 2011). The Hg content of food plants grown in chlor-alkali areas were in the range 0.032-0.59 mg/kg DW (Kabata-Pendias, 2011). These authors also set the toxic Hg content in crop leaves within the range 1-3 mg/kg DW.

Overall, our dataset confirmed the limited translocation of TE in nettle biomass, as compared to woody species grown on the same plots, which indicate that fibres from nettle grown at these sites may be used without restriction in industrial processes.

3.2 Fresh biomass and straw yields

Nettle bales were weighed just after harvesting to determine the yields of fresh matter (Table 3). During the first year, only the experimental site of Tavaux (S1) was harvested at the end of summer. Relatively low yields were measured on each plot and were comprised between 0.293 and 0.817 $t.ha^{-1}$ for fresh matter, and between 0.028 and 0.091 $t.ha^{-1}$ for dry stems. This disparity between each plot and particularly for I3 was due to the presence of plant species (*Galium aparine* L., *Sambucus ebulus* L., *Calystegia sepium* L.) which are able to compete with nettle (Strutek and M, 1998), and also to differences in the quantity of incident light (Yung L et al.). These yields values were lower than those mentioned in the literature (Table 4, (Bacci et al., 2009; Bredemann and Garber, 1959; Dreyer et al., 1996; Francken-Welz et al.; Kôhler et al., 1999; Lehne et al., 2001; Meirheage, Mars 2011; Rexen, 2002; Schmidtke et al., 1998; Vetter et al., 1996) which were in the range 1 - 11.5 $t.ha^{-1}$ for dry stem in the context of traditional agronomic practices. The second year, at the beginning of the summer, two plots (TS and FS) located at the edge of the poplar coppices were harvested in Tavaux (S1) and Fresnes-sur-Escaut (S2) respectively. Yields in fresh matter were 4 to 10 times higher than those measured under the poplar coppices (Table 3). A new weighing was made, after 4 months of air-drying. The dry biomass yield reached a value of approximately 2 $t.ha^{-1}$. The dry stem yields were also measured after leaf removal. The obtained values varied between 0.55 and 1.38 $t.ha^{-1}$, as a function of the harvesting site.

These yields were comparable to the lower values reported in the literature in the context of traditional agronomic practices. Indeed, literature (Bacci et al., 2009; Dreyer et al., 1996; Francken-Welz et al.; Hartl and Vogl, 2009) reported dry matter yields comprised between 2.3 to 15.4 $t.ha^{-1}$ depending on the fertilization conditions. The yields obtained in our site conditions were nevertheless extremely interesting since, in this agro-forestry system, nettle grows naturally and spontaneously, without chemical inputs. The savings associated to the lack of planting / sowing, crop maintenance and inputs may motivate harvesting lower yielding plots.

The fraction of leaves consisted in approximately 35 to 60% of the dry biomass, depending of the year and localisation of harvest. reported leaf fractions around 7%, which emphasizes the differences that can be obtained between the spontaneous nettle and cultivars.

Figure 5 represents the statistical distributions of the stem features for each harvested plots. The features measured for the Tavaux (Fig.5a) and Fresnes-sur-Escaut (Fig.5b) sites show that the length or the diameters of the stems were higher for nettle growing at the edge of the poplar coppice (Y2-S1-TS and Y2-S2-FS) than under the coppice. The mean length was approximately 1.14 m at the edge of the coppice and 0.87 m under poplars. These values of native nettle length were 33 to 50% lower than those measured by (Bacci et al., 2009) (1.7m) for a cultivar using traditional practices.

We further estimated the diameter of the nettle fibres for the plots Y2-S1-TS and Y2-S2-F, with average values of 37 ± 11 and 34± 12 µm, respectively. These values are in agreement with the mean diameters measured by others authors (Di Virgilio, 2013) for nettle cultivars (20-47µm). On the other hand, the thickness of the fibre wall was very different between the two batches. The batch Y2-S2-FS presented an average wall thickness of 6.5 ±2, as compared to a thickness of 12.5 ±3 µm for the Y2-S1-TS batch. Figure 6 shows transverse cross-sections of nettle stems harvested in the second year that are representative of each site, S1 (Fig. 6a) and S2 (Fig. 6b), respectively. It can be observed that the nettle bast fibres presented a quite large lumen area for the two batches, when compared to other European crops such as hemp and flax. For the spontaneous nettle, the harvesting time was not selected as a function of a specific maturity stage. However, it is well known for other crop fibres that the harvest time strongly influences the fibre yield and the fibre filling level (Goudenhooft et al., 2018; Musio et al., 2018). So, the influence of the harvest time of the nettle fibre features needs to be investigated in further studies.

3.3 Continuous fibre extraction using the “all fibre” extraction device

From the standard settings described in section 2.5, the “all fibre” extraction device was able to extract the textile fibres contained in the nettle stalks, leading to the separate production of a fibre-enriched mat (*i.e.* the fibre lap), shives and dust (Figure 7). Shives were conveyed separately via the waste evacuation belt, and dust was removed thanks to the de-dusting fans. The three extracting conditions (C1, C2 and C3) showed significant differences in the quality of the nettle fibres obtained in the outgoing mat. Visually, the C1 condition, corresponding to the use of the as-received nettle stalks, resulted in the cleanest and the finest fibres obtained, with extracted bast fibres up to 10 cm in length.

The material balance of the three trials conducted is mentioned in Table 5. Firstly, it systematically showed a slight decrease in the moisture content of nettle fibres from the inlet to the outlet, which was very likely explained by the use of the de-dusting fans. Moreover, these fans allowed a suitable elimination of dust generated during the fibre extraction (2.2-5.0% of the mass of nettle stalks processed), in priority at the level of module 1 and, to a lesser extent, at the level of the two following modules. Depending on the extracting condition, the fibre lap collected at the outlet of the Cadette machine represented between 11 and 48% of the incoming mass. The C2 samples (*i.e.* straw re-moistened before extraction) had the greatest proportion of fibre lap.

However, this mass proportion must be corrected by the content of impurities inside the fibre lap (Table 5). The latter was estimated inside the three laps produced, impurities corresponding to (i) shives trapped inside the mat, (ii) dust not properly removed by aspiration, and also (iii) the few fibres not yet separated from shives. The content of impurities was minimal (18%) for the C1 condition (control sample), whereas it doubled for the C2 condition (re-moistened stalk) and reached a particularly high value for the C3 one (stalk pre-treated with soda before mechanical extraction).

In conclusion, the as-received nettle straw (*i.e.* control sample, C1) undoubtedly appeared as the most promising raw material, combining (i) the lower impurity (shives) content inside the fibre lap (18% in weight) and (ii) the finest extracted fibre bundles, some of them reaching about

10 cm in length. For this sample, the bast fibres contained in the outgoing mat represented 9.1% of the initial mass of nettle stalks introduced. Before any further textile step (carding), it would still be necessary to clean this mat to remove impurities (shives, dust) trapped between the extracted fibres. For future experiments, such operations could be performed using an additional sieving step for the fibre mat. This nettle fibre yield obtained for the Y2-S1-TS batch is in accordance with the literature, which reports nettle fibre yields between 3 and 16% (Table 4).

3.4 Mechanical properties of fibres

Figure 8 shows the stress/strain curves obtained from single fibre tensile tests, for three different batches that comprised from 32 to 50 fibres. On Figure 8a, results concern nettle fibres from the Fresnes-sur-Escaut site, manually extracted. Figures 8b and 8c presente the results obtained for the nettle fibres obtained on the Tavaux site after manual extraction and after mechanical extraction, respectively. The mean value of the stress at failure for each batch is represented by a dotted line. A large scattering in the response was observed, which is typical of plant fibres (Bourmaud et al., 2018). A significant non-linearity could be observed for most of the tested nettle fibres, which contrasts with the results of Bodros and Baley (Bodros and Baley, 2008) who reported a linear response for nettle fibres manually extracted from retted stems. Table 6 synthetises the mean tensile properties for each batch.

Influence of the harvesting site

The average properties, presented in Table 6 (tensile strength, strain at failure and apparent rigidity), of the nettle fibres extracted manually are equal to 711 MPa, 1.37%, 53 GPa and 812 MPa, 2.14%, 36 GPa for Y2-S2-FS and Y2-S1-TS respectively. These values are lower than those reported in the literature for native nettle cultivars (Bodros and Baley, 2008; Lanzilao et al., 2016). These differences can be due to several factors such as the harvesting time or related to the fibre preparation. If the strength was quite similar for the two tested batches, a significant difference could be observed for the strain at failure and the apparent rigidity. Y2-S1-TS nettle fibres had a mean strain at failure 1.5 time greater than the Y2-S2-FS nettle fibres and an apparent rigidity 1.5 time lower.

This difference in apparent rigidity can be attributed to the difference in the average fibre wall. It was above-mentioned that the wall thickness of the Y2-S2-FS nettle fibres was twice higher than for Y2-S1-TS. The cross-section area (CSA) of the fibres was calculated considering the fibre cylindrical and by neglecting the lumen area. This clearly led to a significant over-estimation of the CSA and then an under-estimation of the apparent rigidity and strength. This under-estimation was more important when the lumen area was large. To ensure a more reliable comparison, additional tests taking into account the real CSA of the nettle fibres are necessary.

Influence of the fibre extraction method

The impact of the extraction mode (mechanical or manual) was also evaluated on the Y2-S1-TS batch. An analysis of variance (ANOVA) was realised on the different values. While the impact on the stress at failure was not statistically representative (p-Value=0.258), a strong influence was observed on the apparent rigidity and on the strain at failure. Interestingly, we can observe a significant difference also in the diameters of the tested nettle fibres, with means of 35 and 28 µm, measured for the tested nettle fibres obtained after manual extraction and after mechanical extraction, respectively. This difference could be attributed to the better fibre separation achieved with the mechanical extraction. The fibre individualisation is a particularly difficult step when made by hand, which is confirmed during the post-treatment of the tensile tests. Using the introduced criterion, it is observed that the bundles of nettle fibres represented approximately 6% of the tested fibres after mechanical extraction against 30% for the manual extraction. For bundle of fibres, a lower rigidity and strength were generally measured (Placet et al., 2017). This was due to the weakness of layer between the individual fibres. So, based on this reasoning, it could be expected to measure a higher stress at failure for the mechanically processed batch. This potential positive effect induced by the improvement of the fibre individualization can also be counterbalanced by the fact that mechanical extraction also generally induces damages and thus a lowering of the properties at failure (Ouagne et al., 2017) .

Finally, the properties obtained for nettle after mechanical processing are promising when compared to the best results obtained for the other European fibre crops, such as flax (see Table

6). (Bensadoun et al., 2017) reported 57 GPa and 791 MPa for the tensile rigidity and strength of industrial flax fibres, respectively, when determined using single fibre tensile test. It is also important to emphasize that for this spontaneous nettle fibres, the harvest time was not optimized and, thus, an improvement of the tensile properties can be expected by adjusting the harvest time to the fibre maturity. In this work, we only considered the apparent properties, which are clearly under-estimated when considering the large lumen area of the tested fibres. By taking into account the real CSA, the effective properties of the fibres could be determined.

4 Conclusions

This work focused on the sustainability of spontaneously stinging nettle grown on trace-element contaminated soil. Our results demonstrate that the use of trace-element contaminated soils is a relevant option to expand the material purpose-grown biomass and at the same time mitigate the land-use conflict between the needs of food and the increasing demand for plant based fibre raw materials.Trace-element contents in nettle fibres are far below the tolerable threshold for agronomic crops. Tensile properties obtained after an extraction made with an “all fibre” industrial extraction device and without field retting or prior alkaline pre-treatment are comparable to the properties of the best industrial flax fibres.

Our set of results is promising in view of a material use. Indeed, the savings associated to the lack of planting/sowing, crop maintenance and inputs may motivate harvesting lower yielding plots. The question of the mechanization of the harvesting, collecting and transportation and thus of the economic balance are however still opened, as well as the generalization of the results to other phytomanagement sites and configurations.

5 Acknowledgements

The authors gratefully acknowledge the funding provided by ADEME, France, under grant n°1772C0018, PHYTOFIBER project and the Fondation de France and ADEME for their financial support to LY under a PhD grant, VNF (Voies Navigables de France) and Valérie Bert (INERIS) for providing us with access to the experimental site at Fresnes-sur-Escaut. This work has also been supported by the EIPHI Graduate school (contract "ANR-17-EURE-0002")

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Figure 1: Photograph of stinging nettle growing spontaneously and in a prevalent manner under short rotation coppice for phytomanagement of trace elements contaminated soils.

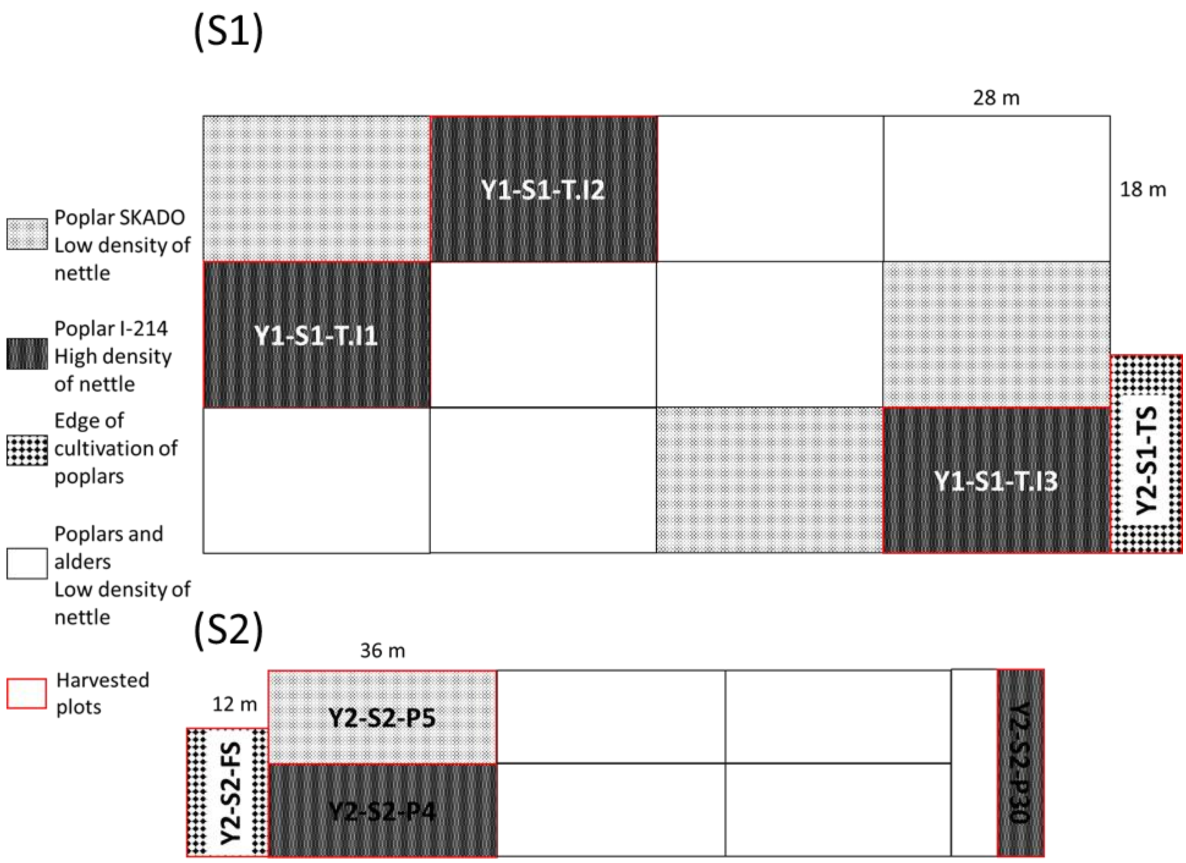


Figure 2: Experimental designs at the two phytomanaged field sites, Tavaux (S1) and Fresnes-sur-Escaut (S2). The sampled plots are in dark grey (I214 plots) and light grey (Skado), on which nettles were collected. Full description of the sites is provided in (Phanthavongsa et al., 2017). Nettles were also collected in the edge of the poplar plots.

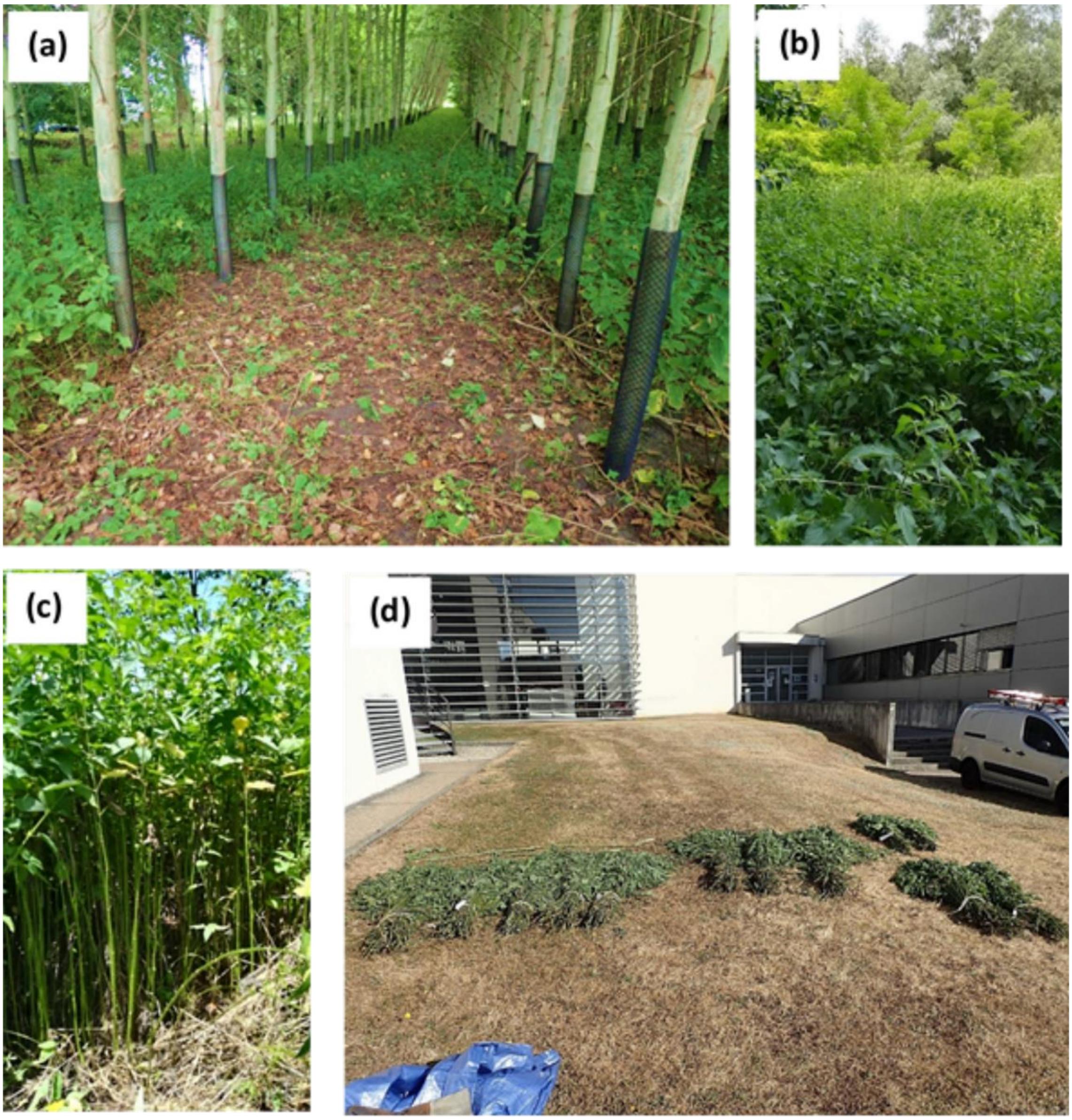
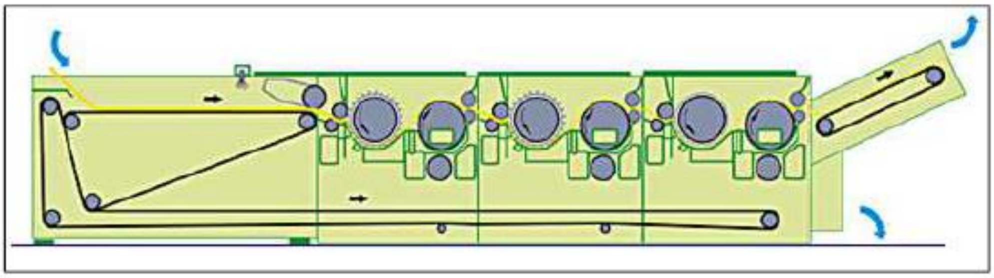
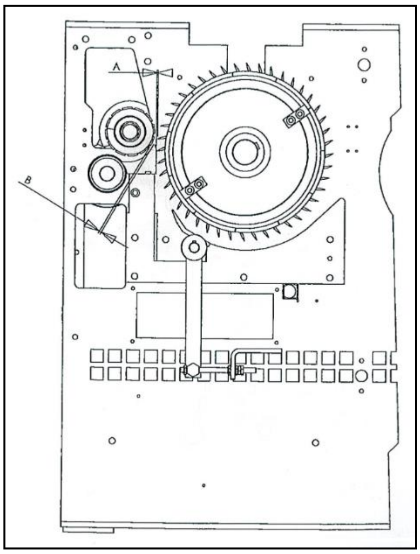


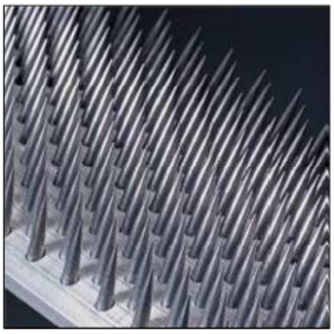
Figure 2: Photograph the plots and the crops. (a) Plot No. Y1-S1-T.I3 after harvest, (b ,c) Nettles in the plot No. Y1-S2-TS, at the wood-edge, and (d) Crop of Y2-S2-(FS, P4, P5, P30).



(a)



(b)



(c)

Figure 3: Laroche (France) Cadette 1000 “all fibre” extraction device used in this study (from Laroche company website). (a) Material path along the opener (in yellow on the drawing), from the feed belt (left) to the exit belt (right). (b) Detailed view (from left to right) of the pair of rolls needed for feeding (smooth roll down and grooved roll up), and the fibre extraction roller (cylinder equipped with nails), all three located at the module inlet. (c) Photograph of the nails of the extracting roller in module 1.

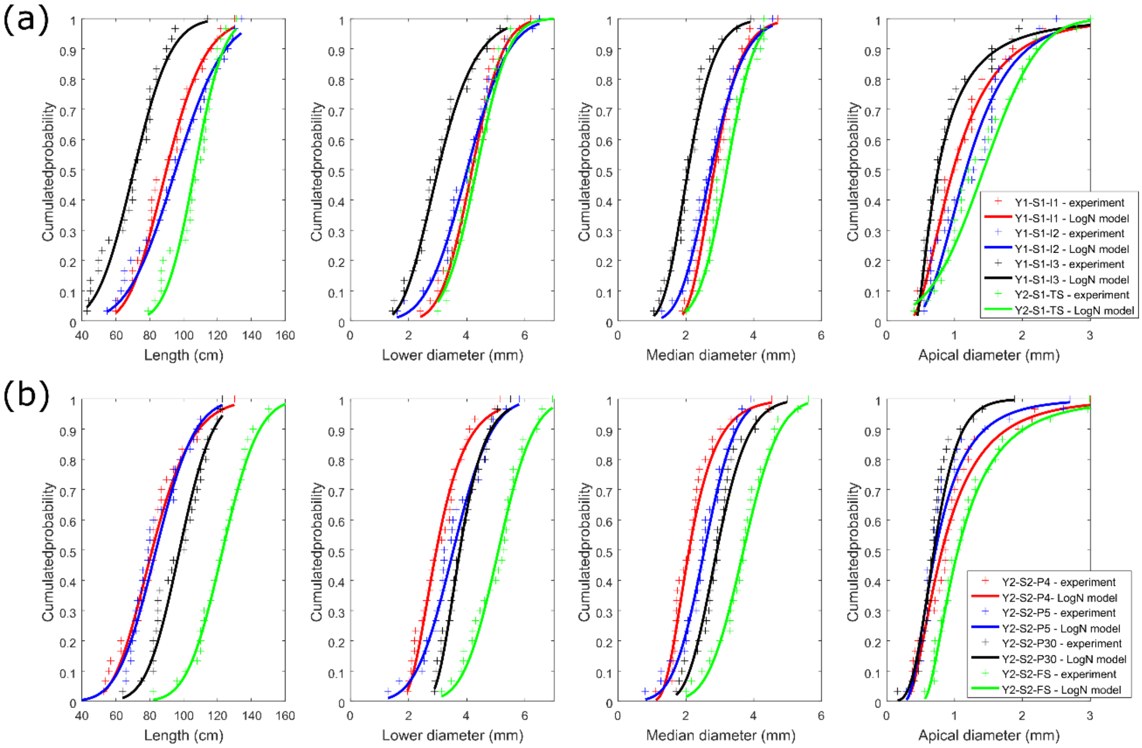


Figure 4: Statistical distribution of the nettle stem features (length and diameter) as a function of the location in the plant and of the harvesting site: S1 (a) and S2 (b).

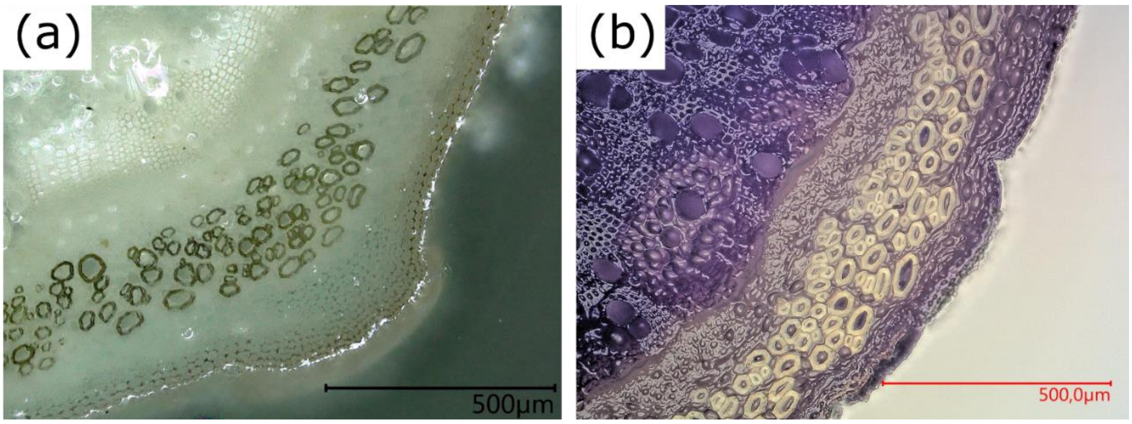


Figure 5: Micrograph of the transverse cross-section of nettle stems. Nettle stems were harvested in second year on S1 (a), and S2 (b) site, respectively.

Figure 6: Pictures of the nettle matters after mechanical processing.

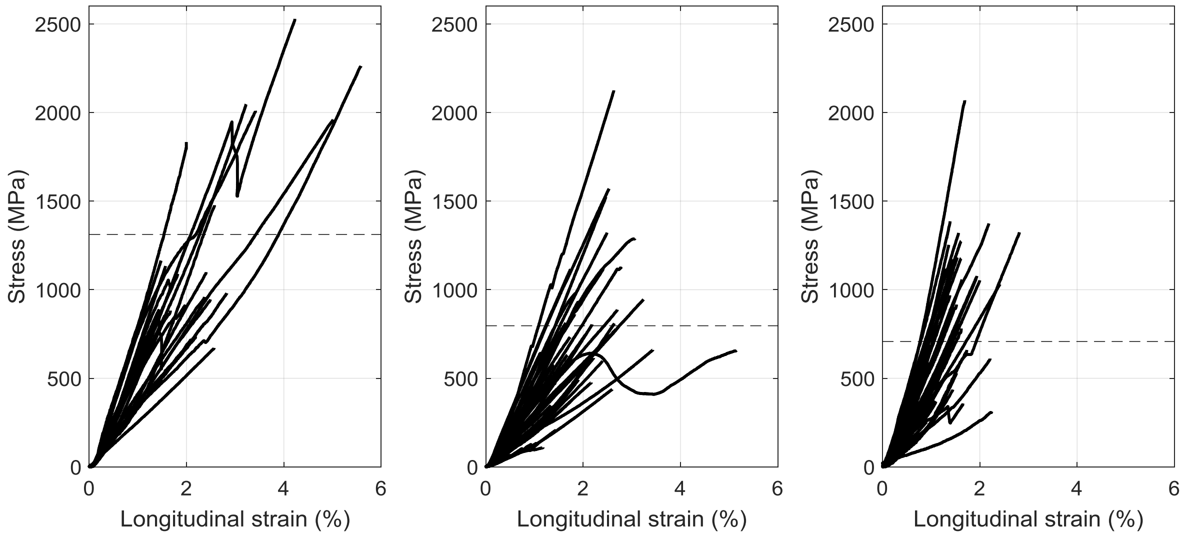


Figure 7: Tensile stress-strain curves of nettle fibres from the batches Y2-S2-FS manually extracted (a), Y2-S1-TS with manual extraction (b) and Y2-S1-TS with mechanical extraction (c).

Table 1: Main meteorological conditions during the growth period at the two sites.

	S1-Y1	S1-Y2	S2-Y1	S2-Y2
Annual average temperature (°C)	11.9	13.1	10.8	11.3
Total annual sunshine (h)	2068	2122	1290	1699
Total annual rainfall (mm)	758	718	543	632

Table 2: Trace element concentrations (mg/kg DW) in soil and nettle samples from the two sediment landfills. For comparison, the TE background concentrations, from natural sites in Belgium were obtained from Tack and Verloo (1996) for Cd, Cu, Pb and Zn, and from our control site located in the East of France for Hg.

Trace element (mg/kg DW)			**Cd**	**Cu**	**Pb**	**Zn**	**Hg**
Industrial sediment landfills			**Fresnes sur Escaut**				**Tavaux**
	Soil (pH = 6.6)	total	15 ± 19	79 ± 33	541 ± 1397	1506 ± 856	6 ± 1
		$CaCl_2$ ext.	0.03 ± 0.01	0.33 ± 0.06	0.13 ± 0.57	0.71 ± 0.003	NA
	Nettle	stems	1.0 ± 0.7	3.3 ± 0.6	1.9 ± 0.4	24.0 ± 6.3	0.01 ± 0.004
	Nettle	fibres	1.1 ± 0.4	2.9 ± 1.0	1.3 ± 0.5	47.5 ± 5.0	NA
	Nettle	leaves	0.9 ± 0.8	9.4 ± 0.53	2.0 ± 0.2	31.9 ± 1.8	0.03 ± 0.01
Control sites			**Belgium**				**France**
	Soil	aqua regia ext.	0.5 ± 0.4	12 ± 5	28 ± 17	54 ± 13	0.6 ± 0.1
	Nettle	leaves & stems	0.4 ± 0.2	14 ± 5	34 ± 19	113 ± 82	0.006 ± 0.003

Table 3: Nettle stem and bast fibre yields (t.ha^{-1}) determined at the two contaminated sites.

	Fresh biomass yield	Dry biomass yield	Dry stem yield
Y1-S1-T-I1	0.73	0.15	0.059
Y1-S1-T-I2	0.817	0.154	0.091
Y1-S1-T-I3	0.293	0.059	0.028
Y2-S1-TS	3.5	-	0.55
Y2-S2-P30	2.95	0.91	0.60
Y2-S2-P4	2.38	0.77	0.34
Y2-S2-P5	2.2	1.01	0.67
Y2-S2-FS	3.76	1.98	1.38

Table 4: Stem and bast fibre yields reported in the literature for fibrescrops cultivated on agricultural lands

	Cultivar	Straw yield ($t.ha^{-1}$)	Bast fibre yield ($t.ha^{-1}$)	References
Nettle	/	3 – 4.1	/	(Bredemann and Garber, 1959)
	/	4.4 – 7.3	/	(Vetter et al., 1996)
	Clone 13	4.88 – 11.52	0.14 – 1.28 (2.9 – 11%)	(Dreyer et al., 1996)
	Clone 13	2.19 – 4.93	0.3 – 0.6 (13.6 – 12%)	(Schmidtke et al., 1998)
	/	2.66 -5.52	0.21 - 0.49 (7.9 – 8.9%)	(Kôhler et al., 1999)
	/	6.71 - 8.12	1.09 - 1.22 (16.2 – 15%)	(Francken-Welz et al.)
	Clone 13	4.4	0.53 (12%)	(Lehne et al., 2001)
	Clone 5-9	2.3 - 9.7	0.3 - 1.02 (13 – 10.5%)	(Hartl and Vogl, 2009)
	/	1 - 10 (3.4 mean)	/	(Rexen, 2002)
	clone 13	15.4	1.696	(Bacci et al., 2009)
Hemp	/	7	2 – 2.2 (28.6 – 31.4%)	(Meirheage, Mars 2011)
Flax	/	6.9	0.7 – 1.7 (10 – 24.6%)	
Oleaginous flax	/	2	0.5 (25%)	

Table 5: Material balance for the three trials conducted, including nettle bast fibre content and impurities level in nettle fibre lap, after mechanical processing

Sample	Control sample (C1)	Moistened (C2)	Soda treatment (C3)
Moisture content at the inlet (%)	10.4 ± 0.2	22.0 ± 0.5	16.5 ± 0.3
Moisture content out of the process (%)	9.6 ± 0.2	18.9 ± 0.3	10.3 ± 0.2
Fibre lap (%)	11.0	48.4	15.4
Shives (%)	86.8	46.6	82.3
Dust (%)	2.2	5.0	2.3
Content of impurities in fibre lap (%)	17.6	36.5	51.0
Real content of bast fibres inside the lap (%)	82.4	63.5	49.0

Table 6: Tensile properties of nettle bast fibres

Mechanical properties of nettle fibres	Y2-S2-FS	Y2-S1-TS	Y2-S1-TS	(Bodros and Baley, 2008)	(Lanzilao et al., 2016)
Fibre extraction	Manual	Mechanical	Manual	Manual	Manual
Diameter (µm)	27.8 ±6.8	28.2 ±6	35.4 ±9	19.9 ±4	24.0 ±15.9
Stress at failure (MPa)	1314 ±552	711 ±427	812 ±451	1594 ±640	2196 ±801
Elastic modulus (GPa)	54 ±17	53 ±24	36 ±19	87 ±28	79 ±29
Strain at failure (%)	2.62 ±1.16	1.37 ±0.53	2.14 ±0.81	2.11 ±0.91	2.8 ±0.9