EFFECT OF FERTILIZATION AND STIMULATION OF *HEVEA BRASILIENSIS* TREES ON MINERAL COMPOSITIONS AND PROPERTIES OF PRODUCED LATEX AND RUBBER.

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Abstract

This publication reports the mineral composition and the properties of latex and rubber obtained from a fertilizer agronomical trial conducted in Kasetsart University Sitthiporn Kritsadakon Research Station, south Thailand. This trial was designed to study the effect of 4 fertilizer levels on RRIM600 clone trees tapped with the recommended tapping system (S/2 d2) with or without stimulation (ET2.5% 4/y). Latex and rubber characterization was conducted on biannual sampling campaigns from 2014 to 2017. Nitrogen (N) and mineral (P, K, Mg, Ca, Ashes) contents were measured on latex and USS rubber made from this latex. Dry Rubber Content (DRC) and Mechanical Stability (MST) of latex as well as Wallace plasticity (P_0), plasticity retention index (PRI) of USS rubber were measured. After 3 agronomical years, latex yield was found to be increased by both fertilization level and stimulation. Concerning N and mineral compositions of latex and USS rubber, no significant effect of fertilization was observed. However, stimulation application was found to be associated with the increase of P content of latex and N, P and Mg contents of USS rubber. Concerning physical properties of latex (DRC, MST) and USS rubber (P_0, PRI), none of these indicators was found to be influenced by either fertilization levels, or stimulant application or their interaction Finally, comparison between latex and USS rubber mineral contents showed that most of P (60%), Mg (82%), and K (89%) was leached in the waste water during the USS making process while the majority of N (34% leached) and the totality of Ca stayed within rubber. The mineral content of the USS process waste water was found to be in the same order of magnitude as the amount brought by fertilizer. Hence, USS process water recycling could be an option to consider.

Keywords *: natural rubber, fertilization, mineral export in latex, mineral loss, rubber sheet process, rubber quality*

INTRODUCTION

Whereas the relation between fertilization and production of latex by mature Hevea trees is not clearly demonstrated and still controversial (Compagnon, 1986 ; Watson, 1989 ; Karthikakuttyamma, 2000), the impact of fertilization on rubber quality is not documented. However, mineral involvement in the structure of the rubber particles and of the rubber material was recently reported (Rippel, 2002 ; Rolere, 2016). Based on an integrated approach (*i.e.* socio-economy, eco-physiology, agronomy, quality) a project was launched in 2014 to document the current practices in Thailand and to set up an appropriate agronomical trial. This trial, conducted in Kasetsart University Sitthiporn Kritsadakon Research Station, was designed to study the effect of 4 fertilizer levels on RRIM 600 clone trees tapped with the recommended tapping system (S/2 d2). Trees tapped at the same frequency but with the application of a stimulant were also studied (S/2 d2 ET2.5% 4/y). Therefore, both stimulation and fertilization effects (and their possible interactions) could be studied. Product quality investigation was conducted on latex and dry rubber on biannual sampling campaigns from 2014 to 2016. Nitrogen (N) and mineral (P, K, Mg, Ca, Ashes) contents were measured on latex and USS rubber made from this latex. Dry Rubber Content (DRC) and Mechanical Stability (MST) of latex as well as Wallace plasticity (P0), plasticity retention index (PRI) of USS rubber were also measured following normalized procedures. This experimental and methodological design aimed to assess the effect of fertilization and stimulation on the produced rubber material, also allowed to evaluate the mineral export by the trees in the latex and the share of this export that is lost in the washing procedure of the USS making process.

MATERIAL AND METHODS

Experimental plot

The experimental plot was located in Kasetsart University Sitthiporn Kritsadakon Research Station, Prachuap Kirikan province, Bang Saphan district (10°59'13.35"N, 99°29'22.41"E). The RRIM600 genotype trees were planted in 2007 (8.1 ha, 3868 trees). The first opening was done in May 2014. The experimental design was an incomplete split-plot design with 4 blocks. Each block contained 8 elementary subplots made by the combination of 4 fertilizers treatments and 2 stimulation treatments as stated in Table 1.

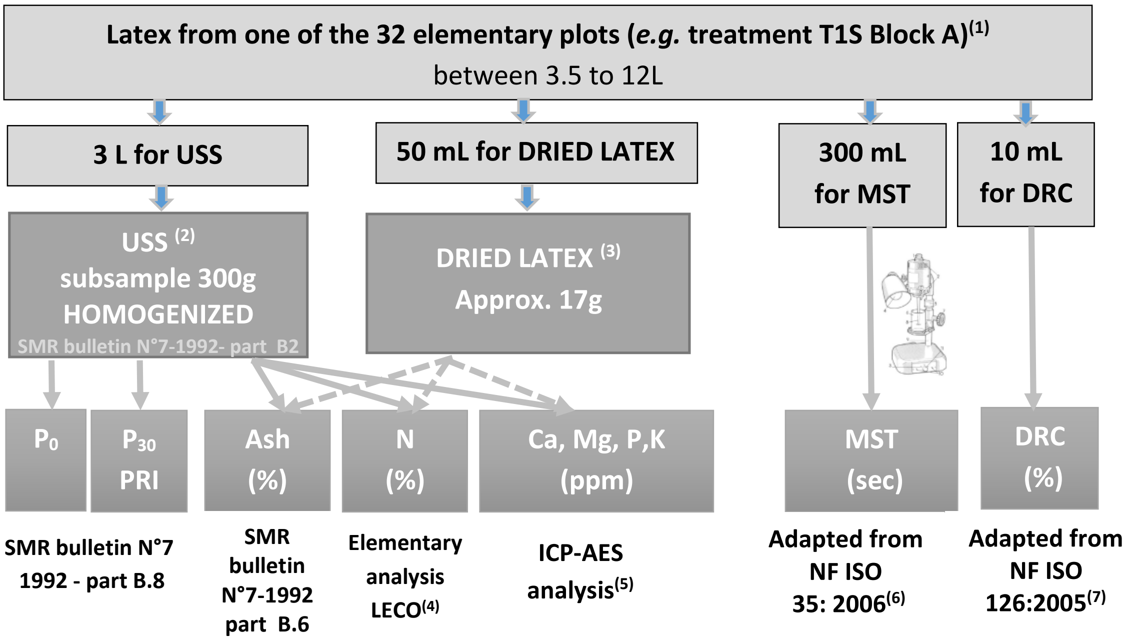
Table 1: Details of the experimental treatments.

Elementary plot	Tapping system		Fertilization (N/P/K)		
				Early rainy season	**Late rainy season**
1	**NS**	S/2 d2 10m(MAY-FEB)/12	**T1**	0	0
2	**NS**		**T2**	500g/tree 15/9/20	0
3	**NS**		**T3**	500g/tree 21/7/14	500g/tree 15/9/20
4	**NS**		**T4**	850g/tree 21/7/14	850/tree 15/9/20
5	**S**	S/2 d2 10m(MAY-FEB)/12 ET2.5% 4/y	**T1**	0	0
6	**S**		**T2**	500g/tree 15/9/20	0
7	**S**		**T3**	500g/tree 21/7/14	500g/tree 15/9/20
8	**S**		**T4**	850g/tree 21/7/14	850/tree 15/9/20

Sitthiporn Kritsadakon Research Station is located at the border between two main climatic regions of Thailand: the equatorial climate region which extends southward from Chumphon province to the border with Malaysia, and the tropical monsoon climate with long rainy season which covers several provinces scattered in the northern, central and the center-eastern parts of the country. The main features of the soil in the experimental plot were a deep soil with a sandy-loam texture and low water retention capacity, a poor organic matter content and cation exchange capacity. Yield was calculated from fresh weight and dry rubber content.

Latex and USS rubber analysis

Starting from October 2014, two sampling campaigns were carried out during each cropping season to collect latex and dry rubber samples for quality analysis. Sampling months were January 2015 - June 2015 – October 2015 – June 2016 – October 2016. Figure 1 summarizes the processing of latex samples collected in each elementary plot and the analytical methods used on both latex and unsmoked sheet rubber (USS rubber).



(1) Latex collection: Tapping is performed from midnight and latex is collected from 4am. Two blocks are collected in one day (16 elementary plots). The two others are harvested under the same conditions in the next tapping day (normally 48h after, if no rain). However, MST is measured on 4 blocks for the 2 collecting days. For each trip, The MST value is obtained from the average of those 2-day measurement.

(2) USS making: USS is made following the RRIT recommendations. 2L of latex is mixed with 3L of water and is added with 300mL of Formic Acid solution 1.56% (94% diluted 60x). The maturation lasted around 45 min. Then the obtained coagulum is manually pressed, then passed 1-2 time(s) to a crusher (final thickness 10 mm), 3-4 times to a flat hand mangle and 2 times to a ribbed handmangle (final thickness 2-3mm). The USS are let dry outdoor under shade (hanging, with daily reversing).

(3) Drying of fresh latex: Within the next two hours after collection, 50 mL of latex are poured in a flat square recipient (22.5 x 22.5 x 3.75 cm) and are placed in a ventilated oven at 70°C until dryness which is assessed visually by the absence of white spot (approx. necessary duration is 24h). Film are collected and conserved in plastic bag containing desiccant bag (5g Silica gel).

(4) Nitrogen analysis (LECO CHN628): a 100mg sample of USS rubber or latex film is wrapped in tin foil cup and is introduced to the furnace via an autosampler. Combustion Furnace temperature is set to 950°C. Calibration was done with EDTA.

(5) ICP AES analysis (Mg Ca P K): 2g of USS rubber or latex film are placed in tared platinum capsule and place in a furnace at 500°C for 2 hours. Ashes are recovered in few drops of water and 2mL of 18% HCl. After 10 min, the solution is filtrated over a ash-free filter. The filtrate is placed in a 25mL volumetric flask while the filter and the solid residue is calcined at 500°C. Ashes are recovered in 2 mL of 40% hydrofluoric acid then evaporated on heating plate. Residue is recovered in 1mL of 18% HCl and then filtrated. The filtrate is added to the first one in same 25mL volumetric flask. The solution is adjusted to 25 mL and is ready to be injected in ICP-AES (Agilent). Wavelengths selected for Mg, P, K, Ca quantification were respectively 285.213, 214.914,404.721 and 430.253 nm.

(6) Mechanical Stability Test: Standard NF ISO35:2006 is followed with the following adaptations: the sample is made of 80mL of fresh latex instead of 80mL of concentrated latex. The latex is not heated and all measurement are done within 2hours after collection.

(7) Dry Rubber Content: Standard NF ISO126: 2005 is followed with the following adaptations: the sample is made of 2mL of fresh latex instead of 10mL of concentrated latex. The volume and concentration of added acid are 15 mL of 2% acetic acid instead of 25-35 mL of 5% acetic acid.

Figure 1 Methodological flow chart for "Quality" assessment of latex and USS rubber samples

RESULTS AND DISCUSSION

1-Latex Yield

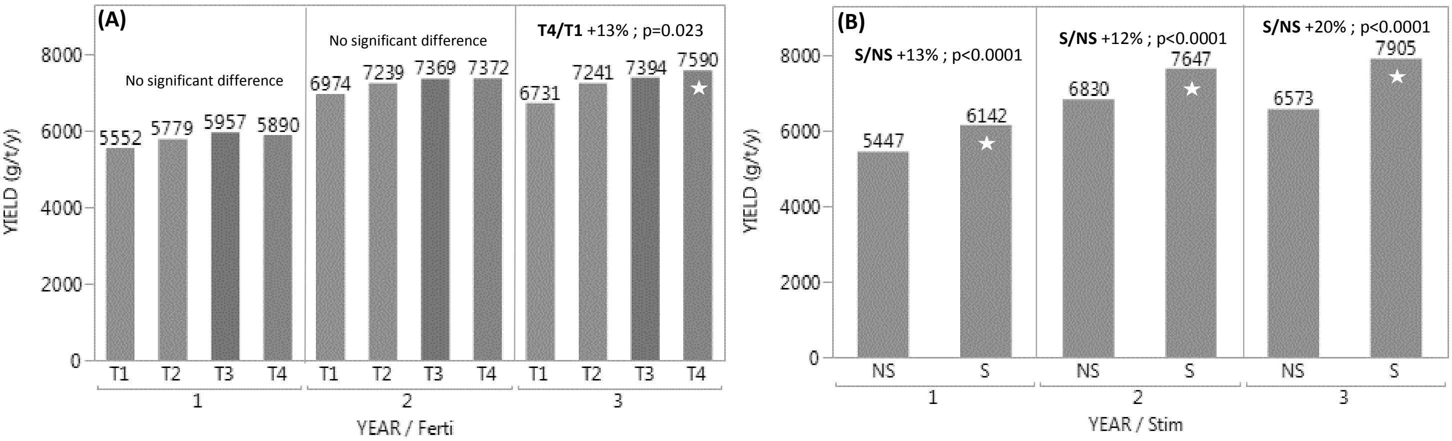


Figure 2 Yield obtained each agronomical year (1: 2015-2015 2:2015-2016 3:2016-2017) averaged by fertilization treatment (A) or by stimulation treatment (B). White stars indicate significant differences (P<0.005) with the control (*i.e.* non fertilized treatment T1 or non-stimulated treatment NS)

The average yield of the plantation (all treatments) was high with nearly 6.0 kg. tree^{-1}. y^{-1} in the first year and more than 7.2 kg. tree^{-1}. y^{-1} in the second and third years which confirmed that the climatic conditions of the experimental site are good for rubber cultivation. Fertilization had a significant effect on yield the third cropping season only though the same trend was observed on the first three years (Figure 2A). However, this figure hides different behavior between stimulated and unstimulated trees. Hormonal stimulation four times a year had a strong significant on yield (Figure 2B). The yield of stimulated trees was 12-20% higher than unstimulated trees.

2- N, mineral compositions and properties of latex and USS rubber

Over the 3-year period, no significant effect of fertilization was observed on the N and mineral composition (Table 2). The same observation remains true when stimulated and non-stimulated trees are observed separately. However, concerning N content of film and latex, the 2016 results (June and October 2016, data not shown) suggest that a positive effect of fertilization on N content could be observed in a longer run observation.

Several significant effects of stimulation application on N and mineral compositions were observed on the 3-year data set:

- For N and Mg this “stimulation” effect is significant on both latex film and USS rubber but not on fresh latex. This could arise from an indirect effect due to the concomitant DRC decrease which is a well-documented effect of stimulation. For an equal concentration expressed versus fresh matter (*e.g.* no difference between Mg content of latex from non-stimulated and stimulated tree), the same concentration expressed versus dry matter would be higher for the “stimulated” treatment because of a lower DRC of the latex. Over the last three years this expected DRC difference was observed for the last 2 October trips (Oct 15 and Oct 16) which correspond to the stimulation period. Figure 3 illustrates this yearly variation for N: DRC evolves inversely compared to the N content expressed versus dry latex (film) or dry rubber.
- For P, the effect is observed on film and fresh latex which means that the physiological concentration in latex increases with stimulation which is consistent with Latex Diagnosis studies that measure inorganic P as an energy level marker sensitive to stimulation. The concentration effect evoked earlier could add up and explains why the prob>F decreases from fresh latex (0.0376) to film (0.0071).
- For ashes, the effect observed in film is consistent with the significant increase of P and Mg. This difference is not conserved in rubber samples.

For the measured parameters, no effect of the interaction fertilization x stimulation was found.

Table 2: N and mineral composition of latex film, fresh latex, and USS rubber for 2014-2017 period (averaged values from 5 sampling campaigns) .

Treatments	STIMULATION		FERTILIZATION			
	Non-Stim	Stim	**T1**	T2	T3	T4
FILM						
N Film_(ppm)	6343	**6620***	6284	6464	6545	6633
P Film_(ppm)	1454	**1558***	1480	1470	1544	1530
K Film_(ppm)	4165	4209	4144	4070	4278	4256
Ca Film_(ppm)	38	38	40	37	39	37
Mg Film_(ppm)	1063	**1164***	1140	1124	1104	1086
Ash Film_(%)	1.47	**1.51***	1.47	1.49	1.50	1.50
FRESH LATEX$^{\Delta}$						
N Latex_(ppm)	2434	2486	2409	2458	2467	2505
P Latex_(ppm)	557	**584***	565	558	581	577
K Latex_(ppm)	1589	1579	1584	1545	1605	1603
Ca Latex_(ppm)	14	14	15	14	14	14
Mg Latex_(ppm)	414	445	444	436	423	416
Ash Latex_(%)	0.56	0.57	0.56	0.56	0.57	0.57
USS rubber						
N USS (ppm)	4706	**4930***	4753	4769	4807	4942
P USS_(ppm)	614	**651***	618	626	644	642
K USS_(ppm)	476	498	467	483	482	517
Ca USS_(ppm)	33	35	35	33	34	33
Mg USS_(ppm)	207	**222***	214	212	210	222
Ash USS_(%)	0.28	0.29	0.28	0.28	0.29	0.28

* significantly different ($P<0.005$) from control (non-fertilized T1 or Non-Stimulated treatment).

$^{\Delta}$ results « fresh latex » have been obtained by calculation from the « film » results. (ppm Fresh Latex = ppm Film x Total Solid Content (TSC) of the latex/100).

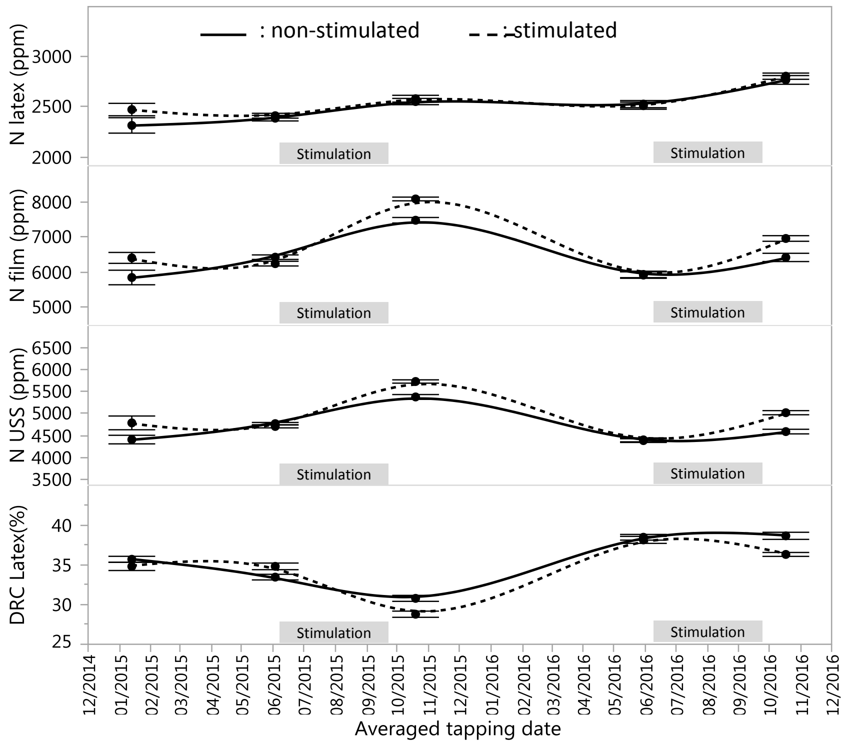


Figure 3: Temporal evolution of N content in fresh latex, dried latex (film) and USS rubber compared with latex DRC evolution (Oct 2014 – Oct 2016) (Error bars illustrate standard error).

3- Properties of latex and USS rubber

Table 3: MST, DRC of latex and physical properties of USS rubber (P_0, P_{30}, PRI) for 2014-2017 period (averaged values from 5 sampling campaigns)

Treatments	STIMULATION		FERTILIZATION			
	Non-Stim	Stim	**T1**	T2	T3	T4
FRESH LATEX						
MST Latex (sec)	57.7	54.4	58.1	57.8	55.7	52.8
DRC Latex (%)	34.7	34.1	34.8	34.4	34.2	34.2
FRESH LATEX$^{\Delta}$						
P_0 USS	39.1	38.6	39.3	39.1	38.5	38.5
P_{30} USS	37.2	37.4	37.2	37.7	37.2	37.1
PRI USS	95.6	97.2	94.9	96.7	97.1	96.8

No significant difference ($P<0.005$)

No fertilization level effect was observed on the measured physical properties of latex and rubber over the 3-year studied period. However, on a single occurrence, a latex destabilization effect of fertilizer was observed in October 15. On this smapling campaign, the mechanical stability of latex harvested from T3 and T4 treatments was found significantly lower than that of non-fertilized treatment (T1).

No effect of stimulation was observed on the measured physical properties of latex and rubber over the 3-year studied period. If the data from October are singled out, this expected decreasing effect of stimulation on DRC appears significant as illustrated in Figure 3.

These absence of global effect of both fertilization and stimulation treatments on the physical properties is a positive result. Indeed, it proves that, so far, none of this treatment has detrimental effect on quality as far as P_0 and PRI are concerned while having a positive effect on produced quantity (increase of yield).

4- Study of the N and mineral loss in waste water during process

The database built during this project allows to estimate the loss rates in USS process waste water by difference between the N and mineral contents of the latex and the corresponding remaining amounts in USS rubber. As an example, N loss rate calculation during USS process, expressed relatively to the initial amount of N in latex, is presented below:

$$\text{N Loss (\%)} = 100*(N_{latex}-(N_{USS}*DRC/100))/N_{latex}$$

or by dividing every terms by the TSC (Total Solid Content)

$$\text{N Loss (\%)} = 100*(N_{film}-(N_{USS}*0.9))/N_{film}$$

Where:

- N_{film} is N content of fresh latex (ppm w/w);
- N_{uss} is N content of USS rubber (ppm w/w),
- DRC is estimated to be equal to 90% of the TSC (DRC= 0.9 TSC)

Figure 4 presents those loss rates calculated from global means for the 5 sampling campaigns (Jan 15 – Jun 15 - Oct 15 – Jun 16 - Oct 16) for all treatments (it was checked that neither stimulation nor fertilization level affects significantly those loss rates).

More than 4 fifths of the ashes were lost in waste water during process of coagulation and lamination in order to obtain USS. The 2 major mineral elements that were lost are K (89%) and Mg (82%), followed by P (60%) and N (34%). No loss of Ca was observed. Ca and, at a lower extent, N, are therefore the analytes showing the strongest interaction with rubber matrix.

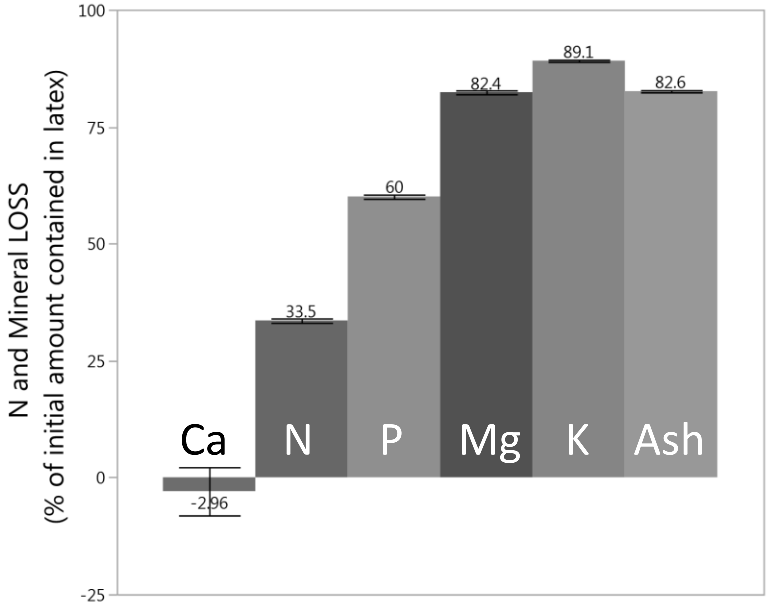


Figure 4: N and Mineral loss rates in USS process waste water (error bar = standard error)

5- Quantitative comparison of fertilizer input with the export in latex and in process waste water.

In this part, N P K amounts brought by fertilizer are compared to the corresponding amounts exuded in latex, a large part of which are discarded in USS process waste water, as shown earlier.

The observed rather large loss rates of N and mineral contents raised the following question: are the N P K loss rates in the same order of magnitude as the quantities brought by fertilizer in treatment T2 to T4? Indeed, if it was the case, recycling of waste water could be an option to be studied. To answer this question a calculation was done and expressed per tree and per year. Table 4 presents for each element (N P K) the elementary amount applied to each tree every year, the elementary amount exuded in the latex produced by that tree in one year, and finally the corresponding elementary amount that is “lost” in the USS process water when this yearly produced latex is transformed in USS rubber. The calculation of the weight of each element exuded in latex by each tree is based on the latex yield and mineral composition per treatment, while that of the weight lost in process water refers to the loss rate determined earlier.

Table 4: N P K weight (g/tree/year) in the applied fertilizer, the produced latex and the process waste water

	Weight **brought by Fertilizer** (g/tree/year)			Weight **exuded in latex** (g / tree /year)			Weight **lost in process water** (g / tree /year)		
	N	**P**	**K**	**N**	**P**	**K**	**N**	**P**	**K**
T1	0.0	0.0	0.0	43.3	10.1	28.5	14.6	6.1	25.5
T2	75.0	19.6	83.0	46.2	10.5	29.1	15.9	6.2	25.8
T3	180.0	34.9	141.1	48.4	11.4	31.5	16.3	6.9	28.1
T4	306.0	59.4	239.9	49.5	11.4	31.7	16.1	6.9	28.1

In order to emphasize the comparison Figure 5 presents normalized data expressed relatively to the initial amounts of N P K brought by each fertilization level.

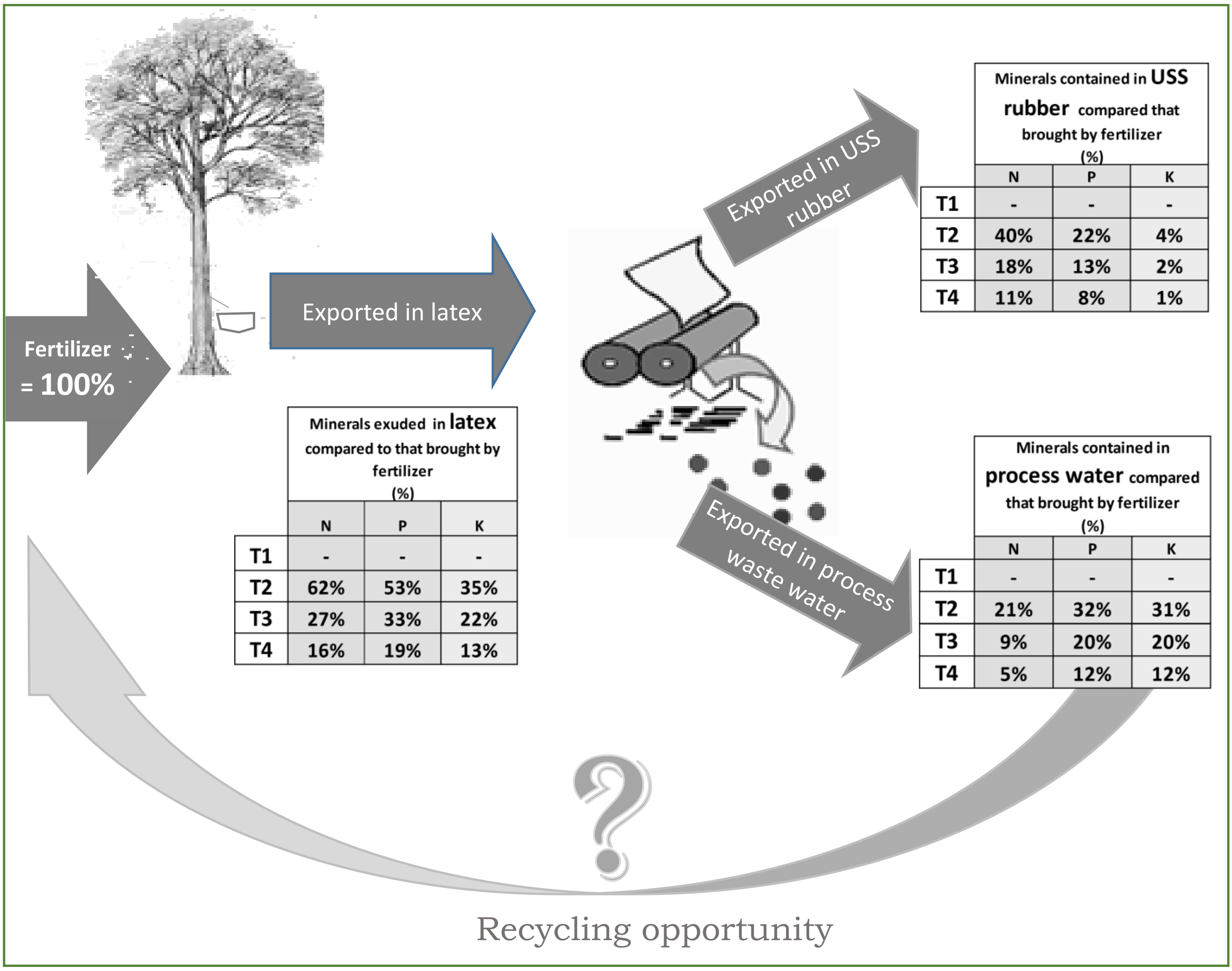


Figure 5: N P K shares in the produced latex and in the process water compared with the amount brought by the fertilizer applications.

If T2 fertilization level is taken as reference, the N amount exuded in latex over one year represent 62% of the amount brought by T2 fertilizer yearly application. Those figures are 53% for P and 35% for K. The amount of N P and K originating from latex and that are released in process water represent respectively 21%, 32% and 31% of the amount brought by T2 fertilizer application. The study of the opportunity to recycle the process water back to the rubber field is therefore to be considered.

Economical and biological studies are necessary to validate such possibility. What is the management cost of recycling amounts of water that are produced every 2 days (frequency of processing corresponds to the frequency of tapping)? Does the organic load of the water (protein, sugar, etc) impairs the recycling possibility, especially the waste water storage? Are the N P K contained in waste water in a form as much absorbable by the tree as it is in fertilizers?

CONCLUSION

Fertilization was not found to affect N and mineral contents (P K Mg Ca) of the fresh latex, film or USS rubber, while stimulation application showed positive influences especially on N, P and Mg contents of film and USS rubber. Neither fertilization nor stimulation was found to significantly affect physical properties of USS rubber made regularly from the latex. Some unconfirmed tendencies has to be observed further by accumulating more data (*e.g.* MST decrease with fertilization, or DRC decrease with stimulation).

Data analysis allowed to calculate the loss of N and mineral content during the USS process. It is shown that more than 4/5 of the ashes were lost in waste water during process. The 2 major minerals that are leached are K (89%) and Mg (82%), followed by P (60%) and N (34%). No loss of Ca was observed.

Those significant figures lead us to compare those "lost" mineral amounts to the quantities that are brought by the proposed fertilizer treatments. It was shown that they are in the same order of magnitude. More than half of the quantity of N and P brought by T2 fertilization level is exuded in latex. Around a third of the amount of P and K brought by T2 fertilization level is contained in the corresponding waste water, which suggest that it could be worth studying if recycling this water back to the field could be profitable.

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REFERENCE

COMPAGNON, P., (1986). Fertilisation. In : Le caoutchouc naturel: biologie, culture, production. G.P. Maisonneuve et Larose. pp. 595.

KARTHIKAKUTTYAMMA, M., MERCYKUTTY, J. et SASIDHARAN NAIR, A.N., (2000) Soils and nutrition. In : Natural Rubber: agromanagement and crop processing. Rubber Research Institute of India. P.J. George and C. Kuruvilla Jacob. pp. 171-198.

RIPPEL, M.M., LEITE, C.A.P., and GALAMBECK,F. (2002) Elemental Mapping in Natural Rubber Latex Films by Electron Energy Loss Spectroscopy Associated with Transmission Electron Microscopy *Anal. Chem.*, 74 (11), pp 2541–2546

ROLERE, S., CHAR, C., TAULEMESSE, J.M., BERGERET, A., SAINTE-BEUVE, J. and BONFILS,F. 2016 The majority of minerals present in natural rubber are associated with the macrogel: An ICP-MS and SEM/EDX investigation J. Appl. Polym. Sci., 133, 43062.

WATSON, G.A., 1989a. Nutrition. In : Rubber. Tropical Agriculture Series. CC Webster & WJ Baulkwill. pp. 291-348.