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Assessing residue effects of tobacco nicotine on the yields, nutrient concentrations and nicotine uptake of a subsequent maize crop

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ABSTRACT

The residue effects of tobacco (*Nicotiana tabacum* L.) nicotine on the subsequent maize (*Zea mays* L.) crop cultivated in soils differing in texture has rarely been studied for over a decade. The present study was conducted on sandy soil of Tabora, and loamy sand soils of Urambo, and Sikonge sites in Tabora region of Tanzania, to assess the residue effects of tobacco nicotine on latter performance of a maize crop. The experiment was laid out in a complete randomized block design with three replications. During the first cropping season (2017–18), the treatments were composed of one unfertilized cultivated plot of tobacco as a control and a fertilized cultivated plot, where the seedlings of flue-cured tobacco variety K326 were transplanted. Two separate plots were kept fallow for planting sole maize (DKC-8053) under fertilized and unfertilized conditions without any nicotine residue during the succeeding second (2018–19) and third (2019–20) cropping seasons. Moreover, in the first cropping season, in one fertilized tobacco plot, tobacco stalks after reaping leaves were incorporated in soil for natural decomposition, while in the other fertilized tobacco plot, tobacco stalks were uprooted. In the second and third cropping seasons, the residue nicotine was measured in soils and found to range from 0.25 to 0.86 mg kg^{-1} in both unfertilized and fertilized tobacco plots. The residue nicotine ranged from 0.92 to 1.05 mg kg^{-1} in fertilized tobacco cultivated soils with tobacco stalks. Results showed that nicotine residue in soil decreased maize grain yield by 0.08 t ha^{-1}. The decrease of maize grain yield was also attributed to inadequate P and K levels in soils planted with tobacco. Therefore, this study recommends the supplemental application of the limiting P and K nutrients when planting maize after tobacco.

1. Introduction

Maize (*Zea mays* L.) is a staple food crop grown by over 95% of Tanzania's small-scale growers (Kassie et al., 2014; Lobulu et al., 2019). Although a large part of the maize crop is consumed in daily diet by most of the smallholder growers, its average production in the country is still low, reaching 2.6 t ha^{-1} against a potential of 4 t ha^{-1} (Nassary et al., 2020). In Tabora region of Tanzania, maize is grown in rotation with tobacco (*Nicotiana tabacum* L.) (Kidane and Ngeh, 2015). Planting crops in a rotation is considered to improve the soil fertility (Shahzad et al., 2016). Maize yield is reported to increase when rotated with diversified crops due to restoration of soil fertility (Parihar et al., 2018). Nitrogen (N), phosphorous (P) and potassium (K) are major nutrients required in balanced amount for significant increase in maize grain (Liu et al., 2006; Setiyono et al., 2010; Laekemariam et al., 2016).

Different authors have different opinions when maize is being raised in rotation with tobacco (Lopez-Lefebre et al., 2001, 2002; Lisuma et al., 2019). Farooq et al. (2014) observed increase in maize growth in terms of improved plant height, number of leaves, dry matter yield and chlorophyll content due to increased soil total N, calcium (Ca), iron (Fe) and zinc (Zn) levels when rotated with tobacco crop. However, in some studies, tobacco cultivation is reported to decrease the levels of K and P in soil (Farooq et al., 2014; Moula et al., 2018; Lisuma et al., 2020a), with no improvement of growth to cereals planted subsequent to tobacco (Yazdani and Bagheri, 2011; Baek et al., 2017). The decreased rates of P and K in soils cultivated with tobacco indicate that tobacco has a genetic affinity for uptake of P and K (Moula et al., 2018; Lisuma et al., 2020a). The insufficient levels of P and K in soils after tobacco may affect the yield of its subsequent maize crop (Anees et al., 2016; Pavuluri et al., 2017), as these nutrients have a specific role in grain filling and to

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final yield of the crop (Liu et al., 2006; Setiyono et al., 2010; Laekemariam et al., 2016).

Research studies conducted on tobacco's influence on subsequent crops are few and these too are associated with the growth effects (Lisuma et al., 2019). To our understanding, the effects of released nicotine in soils through tobacco roots on the subsequent maize grain yield have not been studied yet. The need for finding the effects of nicotine released in the soil through tobacco roots to the subsequent maize crop is significantly crucial as this crop is the primary staple food continuously rotated with tobacco in Tanzania (FAOSTAT, 2016; Lisuma et al., 2019). Therefore, the main objective of the present study was to investigate the effects of residue nicotine levels from tobacco crop on the subsequent maize grain yield in the sand and loamy sand soils. The present study's findings will help farmers decide to plant maize crop after tobacco with supplementation of the limiting nutrients.

2. Materials and methods

2.1. Site description and experimentation

Field experiments were conducted in three sites during the three cropping rainy seasons (2017–2018, 2018–2019 and 2019–20). The sites were Sikonge located at 05° 31′ 47.4″ S and 032° 50′ 03.2″ E with an altitude of 1191 m above sea level (a.s.l.), Tabora located at 05° 03′ 44.4″ S and 032° 40′ 07.4″ E with an elevation of 1160 m a.s.l. and Urambo located at 05° 04′ 33.5″ S and 032° 00′ 09.8″ E at an elevation of 1108 m a.s.l. All experimental fields in each site were under fallow. The sites were selected based on initial survey conducted in tobacco cultivating parts of Tanzania, which represents a global perspective of the tobacco industry. Weather elements in terms of rainfall and temperature were collected during the three cropping periods (Fig. 1) using rain gauge and thermometer respectively.

The experiment was laid out in complete randomized block design with three replications. During the first cropping season (2017–18), the two treatments (T1 and T2) were kept fallow. However, during the succeeding second (2018–19) and third (2019–20) cropping seasons, were planted sole maize under unfertilized (T1) and fertilized (T2) conditions without any nicotine residue. During the first cropping season (2017–18), three separate plots were transplanted with flue-cured tobacco variety K326; unfertilized tobacco (T3), tobacco stalks uprooted after reaping leaves; fertilized tobacco (T4), tobacco stalks were uprooted after reaping leaves and fertilized tobacco (T5), tobacco stalks were incorporated in soils for natural decomposition after reaping leaves. To study the residue effects of nicotine on subsequent maize in tobacco plots, during the succeeding second and third cropping seasons, these three plots were planted with unfertilized maize (T3) as a control, and fertilized maize for T4 and T5.

Tobacco variety K326 (Flue-Cured Tobacco) seedlings were transplanted on ridges spaced 1.2 m apart maintaining a plant to plant distance of 0.5 m in a net plot size measuring 6 m × 6 m with interblock and interplot spacing of 2 m and 1 m, respectively. Maize seed variety Dekalb brand (DKC-8053) was used in the second and third cropping seasons using the same spacing as that of tobacco. Two maize seeds were sown per hole and thinned to one seedling two weeks after seedling emergence. Basal application of $N_{10}P_{18}K_{24}$ fertilizer at 30 g per plant (equivalent to 50 kg N ha^{-1}, 90 kg P ha^{-1}, and 120 kg K ha^{-1}) was done seven days after transplanting tobacco seedlings in November. Two weeks thereafter a top dressing with 8 g calcium ammonium nitrate (CAN 27%) per tobacco plant (equivalent to 34 kg N ha^{-1}) was also done. Subsequent maize crop was fertilized (23:10:5 +3 S+2MgO+0.3Zn) with 86.25 kg N ha^{-1}, 37.5 kg P ha^{-1}, and 18.75 kg K ha^{-1} using a source of YARAMILA CEREAL fertilizer. The N was applied in three equal splits at two, four and seven weeks after sowing maize in November. Maize crop was harvested in March while for tobacco crop leaf started harvested at the end of January and completed in April during the whole study.

2.2. Physical and chemical properties of the soils from experimental sites

The physical and chemical properties of the soils from experimental sites are presented in Table 1. The description of the soil nutrients was based on the compilation of Landon (1991). The soils from Tabora are categorized as sandy, Urambo and Sikonge soils are categorized as loamy sand. Tabora soils were strongly acid (5.49), while Urambo (5.87) and Sikonge (5.89) soils were medium acid. The levels of magnesium (Mg), Ca, and organic carbon (OC) were low, medium K and sulfur (S),

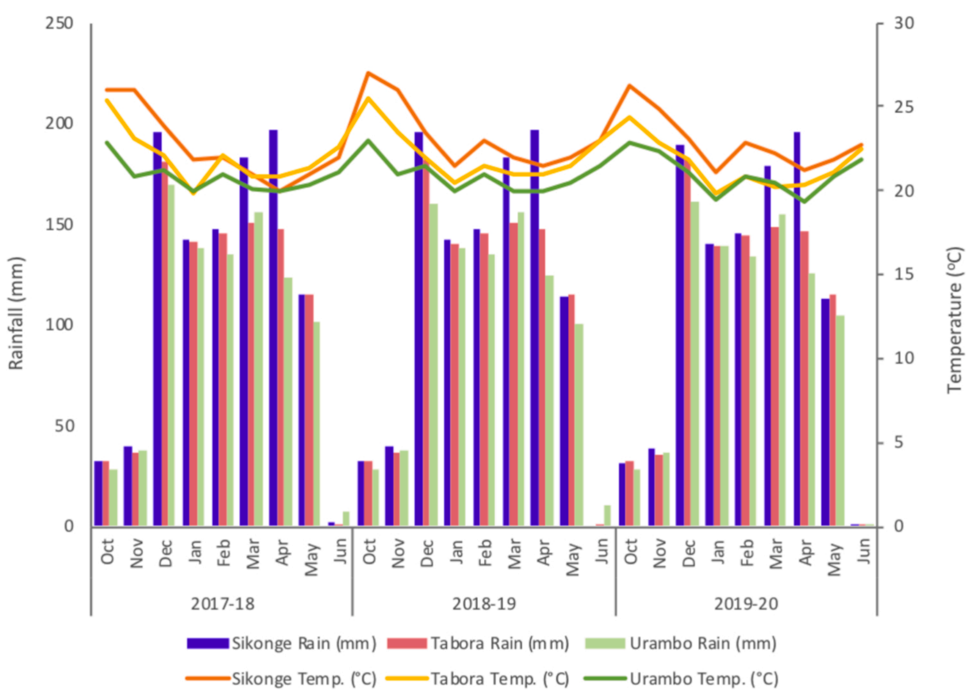

Fig. 1. Mean monthly rainfall (bars) and temperature (lines) of the study sites during the 2017–18, 2018–19 (Lisuma, 2020) and 2019–20 cropping seasons.

Table 1
Physical and chemical characteristics of soils from Tabora, Urambo and Sikonge. The values are before the start of experiment.

Parameters	Sites		
	Tabora	Urambo	Sikonge
pH (1:2.5) in H_2O	5.49	5.87	5.89
Texture Class	Sand	Loamy sand	Loamy sand
Ca	0.10 $cmol_{(+)}$ kg^{-1}	0.40 $cmol_{(+)}$ kg^{-1}	1.29 $cmol_{(+)}$ kg^{-1}
Mg	0.24 $cmol_{(+)}$ kg^{-1}	0.26 $cmol_{(+)}$ kg^{-1}	0.29 $cmol_{(+)}$ kg^{-1}
K	0.29 $cmol_{(+)}$ kg^{-1}	0.25 $cmol_{(+)}$ kg^{-1}	0.53 $cmol_{(+)}$ kg^{-1}
CEC	2.60 $cmol_{(+)}$ kg^{-1}	3.20 $cmol_{(+)}$ kg^{-1}	4.40 $cmol_{(+)}$ kg^{-1}
P	53.39 mg kg^{-1}	44.41 mg kg^{-1}	43.48 mg kg^{-1}
S	8.09 mg kg^{-1}	8.19 mg kg^{-1}	9.12 mg kg^{-1}
N	0.04%	0.04%	0.05%
OC	0.16%	0.25%	0.36%
Nicotine	n/d	n/d	n/d

Source: Lisuma (2020)

very low N, and high P with no nicotine detected (n/d).

2.3. Data collection: Soil and plant analysis

Composite soil samples from 0 to 30 cm soil depth were collected from the experimental plots for determining the texture, pH, OC and other nutrients in the soil. For determining pH, soil water ratio of 1:2.5, as reported by Moberg (2000) was used. The OC was determined using Walkley Black method, total N using Kjedahl method (Bremner and Mulvaney, 1982) and extractable P was determined using Bray 1 method since the soils were acidic in reaction (pH < 7.0) (Moberg, 2000). The exchangeable Ca and Mg in 1 M neutral ammonium acetate (NH_4OAc) filtrates were measured by atomic absorption spectrophotometer (AAS) and exchangeable K in flame photometer (Moberg, 2000). Extractable sulfur (S) was determined by Turbidimetric method (Moberg, 2000). Nicotine was determined in these soils by spectrophotometric analysis using UV visible single beam fixed at 602 nm (Figueiredo et al., 2009). In the second and third cropping season before planting maize, composite soil samples to the depth of 30 cm were collected to determine nicotine residues.

Soil samples were also collected during the second and third cropping seasons before sowing maize as a subsequent crop from the plots with incorporated tobacco stalks and uprooted tobacco stalks to determine soil pH and nicotine levels. A total of nine maize flag leaves from different plants in three middle rows were sampled after the tasseling stage. Maize leaves were air dried at 25 °C to reduce the moisture content before oven drying it at 65 °C to obtain a constant weight. Leaf samples were dried, chopped and ground to pass through a 2-mm sieve and subjected to dry ashing to determine the metals except for S, which was determined by wet digestion (Moberg, 2000). The exchangeable bases Ca^{2+} and K in 1 M neutral NH_4OAc filtrate were measured using an atomic absorption spectrophotometer (AAS) and a flame photometer, respectively (Moberg, 2000). Sulfur (S) was determined by the Turbidimetric method (Moberg, 2000). Total N in the plant samples was determined using the Kjedahl method (Bremner and Mulvaney, 1982), and extractable P was determined using ascorbic acid and molybdate methods (Moberg, 2000). The data on the number of days to maize flowering, grain yield at 12.5% moisture, biological yield and harvest index were also recorded.

2.4. Statistical analyses

The data were analyzed through the STATISTICA 8th Edition, StatSoft, Inc., Tulsa, OK, USA. Experimental sites (Tabora, Urambo and Sikonge) were treated as main plots (fallow plots (T1, T2), unfertilized and fertilized tobacco plots (T2, T3), fertilized tobacco with the removal of tobacco stalks after harvesting (T4) and fertilized tobacco followed by incorporating tobacco stalks in ridges after harvesting tobacco leaves (T5) during the 2017–18 cropping season). Fertilizer/tobacco stalks-based treatments as sub-plots based on Split-Plot Design involved unfertilized maize (T1, T3), fertilized maize (T2, T4, T5) during the 2018–19 and 2019–20 cropping seasons. A two-way ANOVA was performed, and the factor effects model is shown in Eq. 1.

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij} \quad (1)$$

Where; μ is the overall (grand) mean; α_i and β_j are the main effects of factors (sites) and fertilizer/tobacco stalks-based treatments as sub plots; $(\alpha\beta)_{ij}$ is the two-way (first-order) interactions between factors; εij is the random error associated with the observation in the *ijth* factors. The effects of significant means were isolated by a *Post-hoc* Tukey's-HSD test at a threshold (Least significant differences of means) of 5% ($P = 0.05$).

3. Results

3.1. Effect of tobacco cultivation on soil pH and nicotine residue in soils at different sites

Soil pH and nicotine residue in soil after harvesting tobacco leaves but before sowing maize differed significantly ($P \le 0.001$) across the sites. The soil pH was highest at Urambo (5.80, 5.82), followed by Sikonge and Tabora with 5.61, 5.63 and 5.46, 5.47 during the 2018–19 and 2019–20 cropping seasons, respectively. Soil pH (Table 2) during both cropping seasons (2018–19 and 2019–20) under unfertilized (T1) and fertilized (T2) maize were statistically similar, but both were significantly better than the tobacco cultivated plots (T3, T4, T5). On the other hand, the soil pH under unfertilized (T3) and fertilized (T4) tobacco with stalks removed were statistically similar, but were significantly better than the fertilized tobacco plots with incorporated stalks (T5).

Table 2
Effects of tobacco cultivation on soil pH and nicotine residue.

	Cropping season 2018–19[L]		Cropping season 2019–20	
	Soil pH	Soil nicotine (mg kg^{-1})	Soil pH	Soil nicotine (mg kg^{-1})
Site				
Sikonge	5.61 ± 0.06b	0.60 ± 0.14a	5.63 ± 0.07b	0.48 ± 0.12a
Tabora	5.46 ± 0.02c	0.46 ± 0.12b	5.47 ± 0.01c	0.43 ± 0.11b
Urambo	5.80 ± 0.02a	0.27 ± 0.08c	5.82 ± 0.02a	0.24 ± 0.07c
Treatments				
T1: Unf M p fallow	5.75 ± 0.06a	0.00 ± 0.00d	5.75 ± 0.06a	0.00 ± 0.00d
T2: Fert M p fallow	5.76 ± 0.06a	0.00 ± 0.00d	5.77 ± 0.06a	0.00 ± 0.00d
T3: Unf M p unf Tb	5.58 ± 0.05b	0.32 ± 0.06c	5.59 ± 0.05b	0.25 ± 0.05c
T4: Fert M p fert Tb	5.57 ± 0.03b	0.86 ± 0.10b	5.58 ± 0.05b	0.76 ± 0.09b
T5: Fert M p fert Tb+SI	5.47 ± 0.02c	1.05 ± 0.08a	5.49 ± 0.07c	0.92 ± 0.06a
2- Way ANOVA F-statistics				
Site (S)	92.30***	132.48***	99.00**	71.86***
Treatment (T)	28.50***	678.85***	29.90***	460.84***
S x T	10.70***	26.52***	11.20***	16.30***

Key: Values presented are means ± SE $_{\bar{x}}$ (Standard error of means); ***= significant at P ≤ 0.001;
** = significant at P ≤ 0.01; ns = non-significant. Means in the same category of evaluated
interface sharing similar letter(s) do not differ significantly based on their respective Standard error
(SE) at 5% error rate. M= maize; Tb= tobacco; SI= stalks-tobacco incorporated; p = previous;
Unf.= unfertilized; Fert.= fertilized. Cropping season 2018–19[L] (Lisuma, 2020)

The tobacco plots with incorporated stalks exhibited the lowest soil pH values during both the years of study. There were significant interactions between sites and fertilizer treatments on soil pH (Fig. 2). Sikonge had the highest significant soil pH of 5.90 (2018/19); 5.91 (2019/20) in T3, which did not differ significantly from T1, with a soil pH of 5.89 for 2018–19 and 2019–20 cropping season. The lowest soil pH of 5.28 and 5.30 was recorded in T5 of the Sikonge site in the 2018–19 and 2019–20 cropping seasons, respectively (Fig. 2).

Nicotine residues in soil differed significantly with locations/sites (Table 2). At $P \leq 0.001$, Sikonge soils recorded maximum nicotine residue in their soils during 2018–19 and 2019–20 cropping seasons (0.60, 0.48 mg kg^{-1}), respectively, and it was significantly higher than the residue recorded in Tabora soils (0.46 and 0.43 mg kg^{-1}) which in turn was significantly higher than the nicotine residue recorded in Urambo soils (0.27 and 0.24 mg kg^{-1}). On every location, the nicotine residue in soil during the second cropping season decreased in comparison to the first cropping season. In maize treatments, T1 and T2 had no nicotine residues in soils, while unfertilized tobacco plot (T3) recorded the lowest nicotine residue of 0.32 mg kg^{-1} and 0.25 mg kg^{-1} for 2018–19 and 2019–20 cropping seasons, respectively. In the 2018–19 and 2019–20 cropping season, fertilized tobacco plot (T4) recorded nicotine level of 0.86 and 0.76 mg kg^{-1}, respectively. Significant ($P \leq 0.001$) high nicotine residues of 1.05 and 0.92 mg kg^{-1} were recorded in T5 (fertilized tobacco followed by incorporation of tobacco stalks) in the 2018–19 and 2019–20 crop seasons, respectively. The interactions between sites and treatments for nicotine residue in soils were also found to be significant (Fig. 3). Significant ($P \leq 0.001$) high soil residue nicotine of 1.30 and 1.04 mg kg^{-1} were recorded in T5 at the Sikonge site for the 2018–19 and 2019–20 cropping seasons, respectively. The low significant ($P \leq 0.001$) nicotine residue of 0.14 and 0.08 mg kg^{-1} were recorded in unfertilized tobacco (T3) of the Urambo site for the 2018–19 and 2019–20 crop seasons, respectively. The soils of unfertilized maize (T1) and fertilized maize (T2) plots at all three sites did not have any nicotine residue.

3.2. Effects of residue nicotine and fertilizers application on maize flowering and yield indices

Results on days to 50% flowering, maize grain yields, biological yields, and harvest indices during the second and third cropping seasons are presented in Table 3a. In addition to that, standard deviation and percentage of grain yield decrease are presented in Table 3b. Days to 50% flowering of maize plants in the two cropping seasons ranged from 49.20 to 52.00 and did not differ statistically across the sites. The differences in biological yields recorded at different sites during the second and third cropping season were found to be significant. The Sikonge site recorded the highest biological yield (20.25, 18.21 t ha^{-1}) followed by Tabora (18.46, 16.52 t ha^{-1}) and Urambo sites (17.10, 15.23 t ha^{-1}). Maize grain yields recorded in the second and third cropping seasons also differed significantly ($P \leq 0.001$) across the sites. In the second and third season, the significant ($P \leq 0.001$) larger grain yield was obtained to the tune of 2.79 and 2.59 t ha^{-1} at the Urambo site relative to grain yields of 2.52 and 2.32; 2.36 and 2.18 t ha^{-1} recorded at Tabora and Sikonge sites, respectively. Harvest indices of maize followed the same trend of grain yields during both cropping seasons.

In the second cropping season (Table 3a), grain yield in fertilized maize after fallow (T2) was significantly ($P \leq 0.001$) higher with 3.86 t ha^{-1} than T4 grain yield fertilized maize after tobacco (3.53 t ha^{-1}), a decrease of 8.55% (Table 3b). However, grain yields of maize in plots incorporated with tobacco stalks (T5) declined to 3.21 t ha^{-1}, equivalent to 16.84% (Table 3b). A similar trend was observed for unfertilized maize, after fallow (T1) which recorded 1.13 t ha^{-1} grain yields, while unfertilized maize after unfertilized tobacco (T3) recorded 1.05 t ha^{-1} yield, a decrease by 7.08% (Table 3b). The fertilized maize crop after tobacco crop took less number of days to flower than the fertilized maize after fallow. However, the plots with incorporated tobacco stalks significantly decreased the days to flowering for succeeding maize crop (T5). A third cropping season had a similar trend observed in the second cropping season for grain and biological yields. However, the number of days to flowering increased slightly (Table 3a).

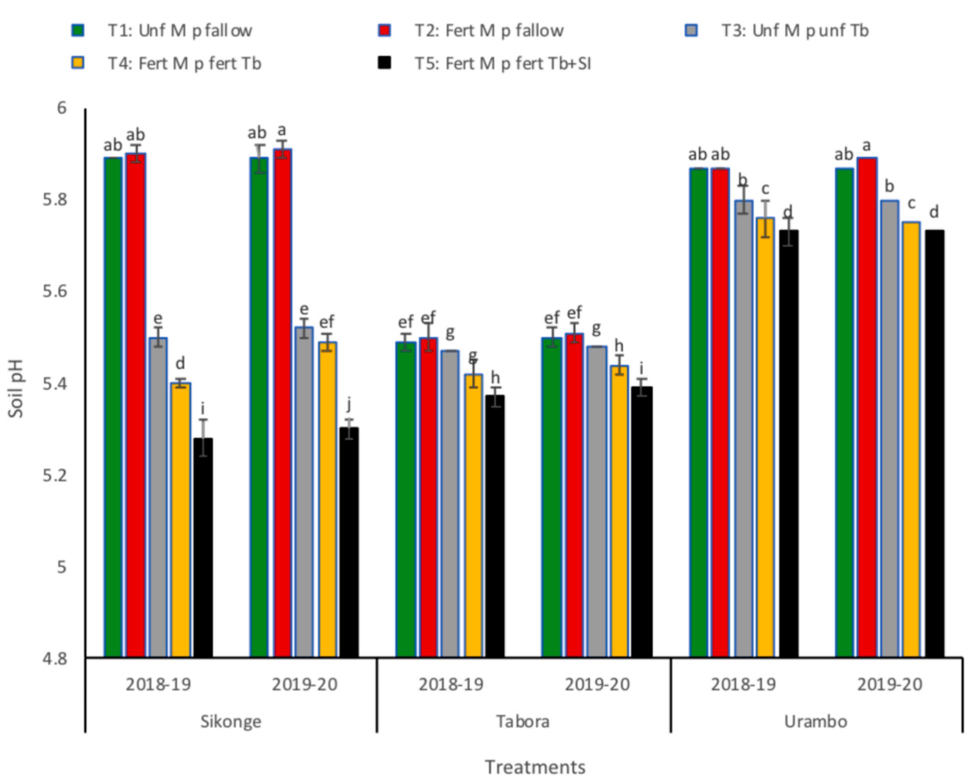

Fig. 2. Interactions of sites and fertilizer treatments on soil pH for 2018–2020. Key: M= maize; Tb= tobacco; SI= stalks-tobacco incorporated; p = previous; Unf = unfertilized; Fert = fertilized.

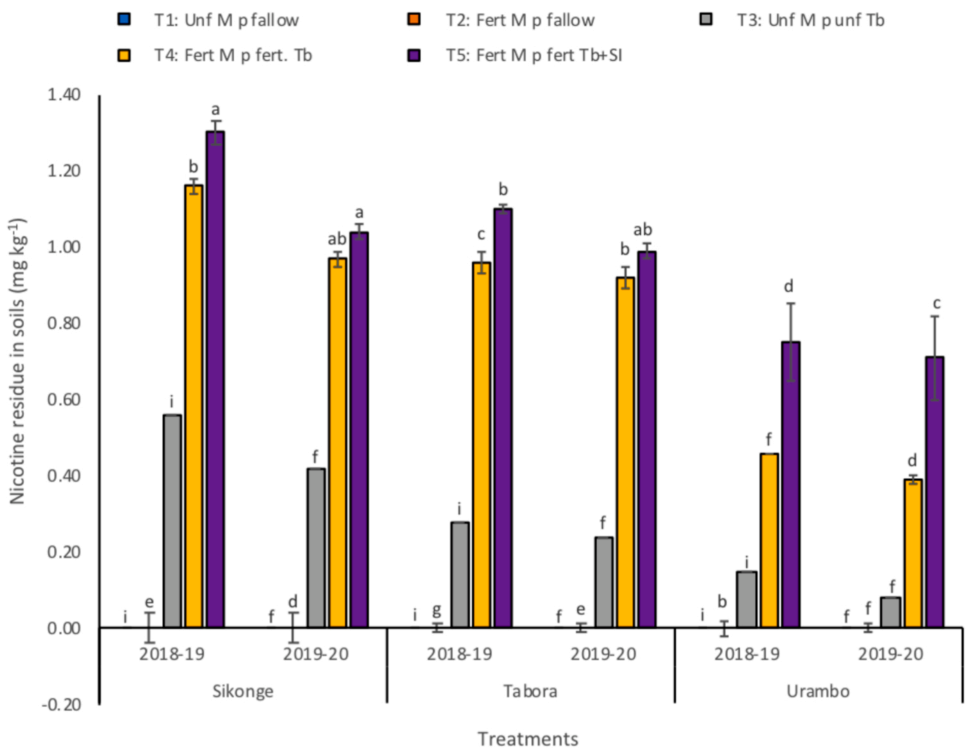

Fig. 3. Interaction effects of sites and fertilizer treatments on soil residue nicotine, Key: M= maize; Tb= tobacco; SI= stalks-tobacco incorporated; p = previous; Unf.= unfertilized; Fert.= fertilized.

Table 3a
Grain yield and yield indices of succeeding maize crop cultivated after tobacco harvest.

	Cropping season 2018–19[L]				Cropping season 2019–20			
	Days to 50% flowering	Grain yield (t ha^{-1})	Biological yield (t ha^{-1})	Harvest Index (%)	Days to 50% flowering	Grain yield (t ha^{-1})	Biological yield (t ha^{-1})	Harvest Index (%)
Site								
Sikonge	50.73 ± 0.99a	2.36 ± 0.32c	20.25 ± 1.18a	11.05 ± 1.23c	51.33 ± 1.00a	2.18 ± 0.30c	18.21 ± 1.11a	11.31 ± 0.01c
Tabora	49.20 ± 3.12a	2.52 ± 0.33b	18.46 ± 1.01b	12.87 ± 1.28b	52.00 ± 0.97a	2.32 ± 0.31b	16.52 ± 0.95b	13.24 ± 0.01b
Urambo	51.80 ± 0.96a	2.79 ± 0.33a	17.10 ± 0.76c	15.55 ± 1.41a	51.93 ± 0.85a	2.59 ± 0.32a	15.23 ± 0.71c	16.12 ± 0.01a
Treatments								
T1: Unf M p fallow	55.00 ± 0.83a	1.13 ± 0.07d	15.02 ± 0.55b	7.61 ± 0.61c	55.77 ± 0.66a	1.01 ± 0.06d	13.27 ± 0.51b	7.77 ± 0.01c
T2: Fert M p fallow	51.55 ± 0.24ab	3.86 ± 0.05a	20.93 ± 0.74a	18.70 ± 0.86a	51.66 ± 0.33b	3.60 ± 0.05a	18.86 ± 0.69a	19.36 ± 0.01a
T3: Unf M p unf Tb	55.55 ± 0.82a	1.05 ± 0.09d	13.96 ± 0.11b	7.57 ± 0.67c	55.11 ± 0.61a	0.94 ± 0.08d	12.27 ± 0.10b	7.73 ± 0.01c
T4: Fert M p fert Tb	47.89 ± 0.42b	3.53 ± 0.12b	21.97 ± 0.95a	16.42 ± 1.13b	48.22 ± 0.22c	3.28 ± 0.11b	19.84 ± 0.89a	16.95 ± 0.01b
T5: Fert M p fert Tb+SI	42.89 ± 4.37c	3.21 ± 0.09c	21.12 ± 0.92a	15.48 ± 0.86b	48.00 ± 0.33c	2.98 ± 0.08c	19.04 ± 0.86a	15.98 ± 0.01b
2- Way ANOVA F-statistics								
Site (S)	0.69ns	20.29***	16.54***	34.54***	1.70ns	20.29***	16.54***	34.13**
Treatment (T)	6.80***	465.81***	57.09***	110.13***	103.20***	465.81***	57.09***	103.70***
S x T	1.12ns	0.83ns	2.41*	2.12ns	4.1**	0.83ns	2.41*	2.07ns

Key: Means in the same category of evaluated interface sharing similar letter(s) do not differ significantly based on their respective Standard error (SE) at 5% error rate. Values presented are means ± SE $_{\bar{x}}$ (Standard error of means); *, **, *** means significant at $P < 0.05$, $P < 0.01$, $P < 0.001$; ns= non-significant ($P \geq 0.05$); M= maize; Tb= tobacco;
SI= stalks-tobacco incorporated; p = previous; Unf.= unfertilized; Fert.= fertilized. Cropping season 2018–19[L] (Lisuma, 2020)

3.3. Nutrient concentrations in maize leaves

Results of the leaf nutrient concentrations in maize for the second and third cropping season are presented in Table 4. In the second cropping season (2018/19), significantly higher leaf nutrient concentrations in maize as an effect of sites were observed on N (2.24%), P (0.26%), K (1.78%), and Ca (0.43%) at Urambo site compared with the concentrations recorded in other sites. While in the third cropping season (2019/20), the same trend observed, with reduced N (1.74%), a slight increase on P (0.27%) and Ca (0.44%), but with an increase on K (3.11%). The individual residue effects of tobacco or its combination with NPK and CAN fertilizers used in maize fields resulted in a significant increase in N and Ca leaf concentrations in the second cropping season but decreased in the third season. A significant increase of

Table 3b
Standard deviation and percentage grain yield decrease of succeeding maize crop cultivated after tobacco harvest.

	Cropping season 2018–19				Cropping season 2019–20			
	Grain yield with no nicotine residue plots (t ha^{-1})	Grain yield on nicotine residue plots. (t ha^{-1})	STDEV	Grain yield decrease due to nicotine (%)	Grain yield with no nicotine residue plots (t ha^{-1})	Grain yield on nicotine residue plots (t ha^{-1})	STDEV	Grain yield decrease due to nicotine (%)
T3: Unf M p unf Tb	1.13	1.05	0.06	7.08	1.01	0.94	0.05	6.93
T4: Fert M p fert Tb	3.86	3.53	0.23	8.55	3.60	3.28	0.23	8.89
T5: Fert M p fert Tb+SI	3.86	3.21	0.46	16.84	3.60	2.98	0.44	17.22

Key: M = maize; Tb = tobacco; SI = stalks-tobacco incorporated; p = previous; Unf.= unfertilized; Fert.= fertilized. STDEV = Standard Deviation

Table 4
Nutrient concentrations in maize leaves.

	Cropping season 2018–19[L]				Cropping season 2019–20			
	N (%)	P (%)	K (%)	Ca (%)	N (%)	P (%)	K (%)	Ca (%)
Site								
Sikonge	2.14 ± 0.14b	0.19 ± 0.01b	1.60 ± 0.10b	0.30 ± 0.01b	1.12 ± 0.15c	0.28 ± 0.00a	1.67 ± 0.12b	0.13 ± 0.01c
Tabora	2.18 ± 0.14b	0.21 ± 0.01b	1.64 ± 0.10b	0.32 ± 0.01b	1.32 ± 0.16b	0.26 ± 0.01a	1.71 ± 0.12b	0.17 ± 0.01b
Urambo	2.24 ± 0.13a	0.26 ± 0.02a	1.78 ± 0.13a	0.43 ± 0.01a	1.74 ± 0.04a	0.27 ± 0.02a	3.11 ± 0.05a	0.44 ± 0.01a
Treatments								
T1: Unf M p fallow	1.33 ± 0.02e	0.19 ± 0.01c	1.65 ± 0.01b	0.28 ± 0.02d	0.94 ± 0.18c	0.28 ± 0.01b	1.91 ± 0.29d	0.25 ± 0.05bc
T2: Fert M p fallow	2.27 ± 0.03c	0.33 ± 0.02a	2.44 ± 0.07a	0.35 ± 0.01b	1.93 ± 0.12a	0.32 ± 0.02a	2.65 ± 0.20a	0.27 ± 0.05a
T3: Unf M p unf Tb	1.90 ± 0.04d	0.19 ± 0.01c	1.11 ± 0.02d	0.33 ± 0.03c	0.89 ± 0.16c	0.26 ± 0.01b	1.70 ± 0.33e	0.24 ± 0.05c
T4: Fert M p fert Tb	2.57 ± 0.02b	0.23 ± 0.00b	1.61 ± 0.02 BCE	0.39 ± 0.01a	1.59 ± 0.13b	0.22 ± 0.02c	2.39 ± 0.14b	0.22 ± 0.04d
T5: Fert M p fert Tb+SI	2.87 ± 0.02a	0.16 ± 0.01d	1.54 ± 0.04c	0.39 ± 0.01a	1.62 ± 0.03b	0.27 ± 0.01b	2.17 ± 0.21c	0.26 ± 0.04ab
2- Way ANOVA F-statistics								
Site (S)	7.72**	26.82**	14.74***	136.06***	28.69***	0.60ns	3051.35***	1717.79***
Treatment (T)	708.46***	60.44***	238.20***	32.62***	35.65***	9.53***	386.36***	13.61***
S x T	1.48ns	7.72***	1.53ns	4.15**	5.06ns	4.19**	61.28***	5.25***

Key: Means in the same category of evaluated interface sharing similar letter(s) do not differ significantly based on their respective Standard error (SE) at 5% error rate. Values
presented are means ± SE $_{x^-}$ (Standard error of means); **, *** means significant at $P < 0.01$, $P < 0.001$; ns= non-significant ($P \geq 0.05$); M= maize; Tb= tobacco; SI= stalks-tobacco
incorporated; p = previous; Unf.= unfertilized; Fert.= fertilized. Cropping season 2018–19 [L] (Lisuma, 2020)

maize's P and K leaf concentrations was observed in fields where only NPK and CAN fertilizers were applied in the absence of tobacco effects in the second and third cropping season. The interactions of sites and tobacco or fertilizer treatments were highly significant on leaf concentrations of P and Ca in the second cropping season, while significant interactions observed for P, K and Ca leaf concentrations in the third cropping season.

Table 5
Nutrients uptake by succeeding maize crop during the two cropping seasons after tobacco harvest.

	Cropping season 2018–19				Cropping season 2019–20			
	N (g plant^{-1})	P (g plant^{-1})	K (g plant^{-1})	Ca (g plant^{-1})	N (g plant^{-1})	P (g plant^{-1})	K (g plant^{-1})	Ca (g plant^{-1})
Site								
Sikonge	12.99 ± 2.84a	1.70 ± 0.25c	15.55 ± 2.02b	2.22 ± 0.27a	11.05 ± 2.48a	2.26 ± 0.33a	13.99 ± 1.81b	1.11 ± 0.17c
Tabora	9.32 ± 1.28b	2.61 ± 0.39b	16.93 ± 3.05a	1.30 ± 0.19c	12.68 ± 2.57a	2.11 ± 0.34a	15.15 ± 2.74b	1.34 ± 0.20b
Urambo	14.90 ± 2.98a	2.88 ± 0.43a	16.76 ± 3.02a	1.52 ± 0.23b	7.99 ± 1.08b	1.21 ± 0.19b	15.01 ± 2.70ab	1.98 ± 0.24a
Treatments								
T1: Unf M p fallow	2.30 ± 0.33c	0.74 ± 0.02d	4.00 ± 0.69e	0.58 ± 0.10e	2.46 ± 0.25c	0.85 ± 0.07c	5.15 ± 0.31d	0.66 ± 0.08d
T2: Fert M p fallow	23.61 ± 2.82a	4.23 ± 0.29a	29.45 ± 1.29a	2.78 ± 0.22a	20.41 ± 2.50a	3.35 ± 0.29a	26.44 ± 1.15a	2.45 ± 0.21a
T3: Unf M p unf Tb	2.88 ± 0.27c	1.09 ± 0.07c	5.77 ± 0.34d	0.75 ± 0.08d	1.86 ± 0.31c	0.56 ± 0.03c	3.57 ± 0.62e	0.51 ± 0.09e
T4: Fert M p fert Tb	17.23 ± 1.81b	2.97 ± 0.37b	24.04 ± 0.93b	2.06 ± 0.21c	14.69 ± 1.57b	2.25 ± 0.35b	21.59 ± 0.83b	1.81 ± 0.20c
T5: Fert M p fert Tb+SI	15.99 ± 1.36b	2.96 ± 0.24b	18.79 ± 0.39c	2.22 ± 0.08b	13.43 ± 1.13b	2.29 ± 0.23b	16.82 ± 0.35c	1.95 ± 0.08b
2- Way ANOVA F-statistics								
Site (S)	10.08***	55.01***	3.39*	244.93***	8.76***	28.81***	2.95ns	275.98***
Treatment (T)	66.61***	181.81***	452.05***	595.66***	60.98***	70.24***	444.85***	588.34***
S x T	3.73**	7.15***	7.28***	14.26***	3.50**	3.51**	7.05***	15.94***

Key: Means in the same category of evaluated interface sharing similar letter(s) do not differ significantly based on their respective Standard error (SE) at 5% error rate. Values
presented are means ± SE $_{x^-}$ (Standard error of means); *, **, *** means significant at $P < 0.05$, $P < 0.01$, $P < 0.001$; ns= non-significant ($P \geq 0.05$); M= maize; Tb= tobacco;
SI= stalks-tobacco incorporated; p = previous; Unf.= unfertilized; Fert.= fertilized.

3.4. Nutrient uptakes by maize

The uptakes of N, P and K by maize plants during the second cropping season were significantly high at the Urambo site (Table 5). However, the uptake of Ca by the maize plant was high at Sikonge. During the third cropping season, N, P, and K were significantly higher at Tabora and Ca at Urambo. The interaction between sites and fertilizer regimes were also significant on the uptake of N, P, K, and Ca by the maize plants (Table 5). Further, the uptakes of N, P, K, and Ca nutrients in unfertilized maize plants sown after the unfertilized tobacco plants (T3) in the 2018–19 cropping season were increased by 0.58, 0.35, 1.77, and 0.17 g plant^{-1}, respectively over the unfertilized maize after fallow (T1). However, in the 2019–20 cropping season, uptakes of N, P, K, and Ca nutrients decreased by 0.6, 0.29, 1.58 and 0.15 g plant^{-1}, respectively when compared with the values obtained under unfertilized maize after (T1). Except Ca uptake, results indicated that release of nicotine from the incorporated tobacco stalks caused a decrease in the uptake of all the other estimated nutrients by the following maize crop (Table 5).

3.5. Nicotine uptakes by maize

The maize plants analyzed for nicotine content in roots, leaves and grain yields differed significantly across the sites (Table 6). During both cropping seasons (2018–19 and 2019–20), the maize roots, leaves and grain yields obtained from Sikonge and Tabora sites were higher in nicotine than those obtained from Urambo site (Table 6). However, the nicotine uptake levels decreased slightly in 2019–2020 compared to the 2018–19 cropping season. Significantly larger quantities of nicotine in maize plants were found in fertilized maize cultivated in previously fertilized tobacco cultivated soils and tobacco stalk incorporated than in previously unfertilized tobacco cultivated soils to both 2018–19 and 2019–20 cropping seasons.

During the 2018–19 cropping season, maize roots took up more nicotine at the range of 7.09–7.43 g plant^{-1} compared with 6.36 – 6.68 g plant^{-1} in the 2019–20 cropping season. The uptake of nicotine in maize leaves reduced to a range of 0.60 – 0.74 g plant^{-1} and 0.37 – 0.47 g plant^{-1} during 2018–19 and 2019–20 cropping seasons, respectively. The amount of nicotine taken up by maize grain was negligible, ranging from 0.013 to 0.016 g plant^{-1} and 0.011 – 0.012 g plant^{-1} to both cropping seasons. In plots where no tobacco was cultivated, the nicotine uptake was still observed in maize roots and leaves which ranged from 0.77 to 1.51; 0.39 – 0.61 and 0.11 – 0.28; 0.03 – 0.11 g plant^{-1} during the 2018–19 and 2019–20 cropping seasons, respectively. However, uptake of nicotine in maize grains was not detected during the both cropping seasons (Table 6).

4. Discussions

Tobacco crop planted in the first crop season (2017–18) released nicotine in the soils and the amount differed across the sites. However, Sikonge recorded the highest soil nicotine levels reaching 0.60 mg kg^{-1} than Tabora and Urambo with nicotine levels of 0.46 and 0.27 mg kg^{-1}, respectively. Nicotine was released more in fertilized tobacco soils ranging from 0.86 to 1.05 mg kg^{-1}, where tobacco stalks were incorporated in soils (Table 2). Increased nicotine levels in soils lowered significantly the soil pH to a range of 5.57–5.47 compared to fertilized and unfertilized plots of maize without any previous crop of tobacco which had soil pH of 5.76 and 5.75 respectively (Fig. 2). The nicotine released in the soils increases acidity, and the acidic nature adsorbs more nicotine through protonation of the pyrrolidine N atom by receiving a H^+ and hence results in the reduction of soil pH due to the H^+ generated (Fig. 3; Rakić et al., 2010; Shakeel, 2014; Lisuma et al., 2020ab).

The nicotine released by the previous fertilized tobacco crop tends to shorten the days to flowering (47.89) of succeeding fertilized crop of maize compared to 51.55 days for the fertilized maize crop with no preceding tobacco crop (Table 3a). Zhou et al. (2014) reported that the released tobacco nicotine in soils improved maize growth. However, the fertilized crop of maize without any preceding tobacco crop (T2) produced higher yield (0.33 t ha^{-1}) over the fertilized maize crop with preceding crop of tobacco whose stalks were removed (T4). The presence of certain amount of nicotine seemed to be the cause in reduced yield (Lisuma, 2020). The incorporation of tobacco stalks released high levels of nicotine and significantly decreased the yields of succeeding maize by 0.32 t ha^{-1} (in T5) over maize plots with preceding tobacco crop without stalks (T4). This indicates that the residue nicotine in soils decreased maize grain yield (Table 3a) due to the low uptake of P and K (Table 5). Consistently, a study by Moula et al. (2018) observed that tobacco nicotine released in soils resulted in reduced P and K levels. On the contrary, the yield from unfertilized maize (T3) after unfertilized tobacco declined by 0.08 t ha^{-1} equivalent to 7.08% (Table 3b) over the unfertilized maize without any preceding tobacco crop. This observation signifies that the effect of nicotine residue in soils, regardless of how low, it has a similar impact on reducing maize grain yield to subsequent maize crop (Lisuma, 2020). Hence, fertilizing the succeeding maize crop after fertilized tobacco crop cause four fold depression in maize yields due to enhanced levels of nicotine residue in soils.

Fertilized maize plants after tobacco crop were observed to grow

Table 6
Nicotine uptake by the succeeding maize during 2018–19 and 2019–20 after tobacco harvest.

	Cropping season 2018–19			Cropping season 2019–20		
	Maize parts (g plant^{-1})			Maize parts (g plant^{-1})		
	Roots	Leaf	Grain	Roots	Leaf	Grain
Site						
Sikonge	4.25 ± 1.04a	0.44 ± 0.08a	0.012 ± 0.000a	3.79 ± 0.94a	0.24 ± 0.06a	0.009 ± 0.003a
Tabora	3.94 ± 0.98a	0.46 ± 0.09a	0.005 ± 0.000b	3.52 ± 0.89a	0.26 ± 0.06a	0.004 ± 0.001b
Urambo	2.17 ± 0.52b	0.20 ± 0.05b	0.001 ± 0.000c	1.94 ± 0.46b	0.12 ± 0.03b	0.001 ± 0.000b
Treatments						
T1:Unf M p fallow	1.51 ± 0.07b	0.11 ± 0.01c	0.000 ± 0.000b	0.39 ± 0.12c	0.03 ± 0.01b	0.000 ± 0.000b
T2:Fert M p fallow	0.77 ± 0.12c	0.28 ± 0.08b	0.000 ± 0.000b	0.61 ± 0.10c	0.11 ± 0.04b	0.001 ± 0.000b
T3:Unf M p unf Tb	0.46 ± 0.13c	0.11 ± 0.01c	0.000 ± 0.000b	1.36 ± 0.06b	0.05 ± 0.01b	0.000 ± 0.000b
T4:Fert M p fert Tb	7.09 ± 0.68a	0.74 ± 0.09a	0.013 ± 0.000a	6.36 ± 0.61a	0.47 ± 0.07a	0.011 ± 0.003a
T5:Fert M p fert Tb+SI	7.43 ± 0.85a	0.60 ± 0.08a	0.016 ± 0.000a	6.68 ± 0.76a	0.37 ± 0.06b	0.012 ± 0.003a
2- Way ANOVA F-statistics						
Site (S)	36.57***	12.45***	15.08***	35.76***	5.77***	12.19ns
Treatment (T)	213.98***	29.89***	20.59***	213.41***	21.14***	14.55***
S x T	8.54***	1.36ns	4.75***	8.51***	1.00ns	5.16***

Key: Means in the same category of evaluated interface sharing similar letter(s) do not differ significantly based on their respective Standard error (SE) at 5% error rate. Values presented are means ± $SE_{\bar{x}}$ (Standard error of means); *** means significant at $P < 0.001$; ns= non-significant ($P \geq 0.05$); M= maize; Tb= tobacco; SI= stalks-tobacco incorporated; p = previous; Unf.= unfertilized; Fert.= fertilized.

faster due to N levels, which recorded significantly higher N leaf concentrations than maize leaf not preceded by tobacco (Table 4). Thus, residue nicotine influenced maize growth as nicotine was observed to be associated with higher molecules of N (Hu et al., 2018). However, maize leaf P and K planted after tobacco had the lowest leaf concentration below the critical maize leaf concentration (Landon, 1984) and uptake (Table 5), which reduced maize grain yields to both cropping seasons as these nutrients are essential for grain filling. Other studies justify that P and K are attributed to the maize yield (Laekemariam et al., 2016; Ma et al., 2016; Kihara et al., 2017; Njoroge et al., 2018). The slight decrease in grain yields during the 2019–20 cropping season could be attributed to the slight decrease in average rainfall and temperature compared to the 2017–18 cropping season. The 2018–19 cropping season recorded better average of rainfall than the rest (Fig. 1). Therefore supplementing K and P nutrients to the maize planted as a subsequent crop to tobacco is required for increased grain yield.

The nicotine uptake in roots of unfertilized maize cultivated subsequent to unfertilized were low (0.46–1.36 g plant^{-1}) in comparison to the nicotine uptake in roots of fertilized maize cultivated subsequently to fertilized tobacco (7.09–6.36 g plant^{-1}) during the 2018–19 and 2019–20 cropping seasons respectively (Table 6). This observation indicates that fertilized tobacco releases high nicotine in soils that are also subject to uptaken by a subsequent maize roots (Lisuma, 2020). The average nicotine uptake in roots under the tobacco-maize rotation (T4) was 6.72 g plant^{-1} (Table 6). In addition, the highest nicotine uptake in maize cultivated preceded by tobacco with tobacco stalks incorporated ranged from 7.43 to 6.67 g plant^{-1} during the 2018–19 and 2019–20 cropping seasons, respectively. However, the nicotine uptake by maize was lowest during the 2019–20 cropping season due to the decomposition of roots and leaf remnant in soils. During both cropping seasons the levels of nicotine detected in leaves were significantly reduced, reaching an average of 0.060 g plant^{-1}. Weidner et al. (2005) observed *Epipremnum aureum* plant root ability in uptaking soil nicotine and transferring it to the shoots. Thus, its nicotine levels in leaves decreased as nicotine levels in soil media decreased. A similar trend of nicotine uptake in roots of maize plants was also observed to account for these processes in soils and leaves.

Interestingly, our study observed that even in maize planted with or without fertilization and/or without incorporation of tobacco residues had some nicotine residues. The nicotine uptake ranged from 1.51 to 0.77 and 0.39–0.61 g plant^{-1} during the 2018–19 and 2019–20 cropping seasons (Table 6). This finding suggests that there may be some nicotine released from the tobacco roots that percolated through water and absorbed by maize roots. Other studies reported that plants growing nearby tobacco plants could absorb nicotine released by tobacco roots (Selmar et al., 2018, 2019; Lisuma et al., 2020b). In addition, decomposed tobacco plants create a possibility to exchange nicotine with newly planted plants and the released nicotine could be adsorbed in the soil (Selmar et al., 2018, 2019).

On the other hand, nicotine was not detected in the maize grain even in maize plants cultivated after tobacco. Only traces of nicotine levels were detected at the maize grain after tobacco crop ranging from 0.011 to 0.016 g plant^{-1}, which were regarded as negligible (Table 6). This indicated that maize plants can physiologically filter foreign substances from entering the maize grain (Singh and Ghosh, 2011; Beri at al, 2020), as nicotine decreased significantly towards the leaf (Table 6).

Maize grain is used as food for humans, whereas it is also used as animal feeds, including roots and leaves (Thorne et al., 2002). Therefore, human being may consume nicotine directly in maize based food and by consuming animal products from animals fed maize containing formulations. However, the detected nicotine level in our study is extremely low to cause any health effects. The nicotine's lower limit i.e lethal death (LD) - 50 causing fatal outcomes is 0.5–1.0 g if ingested, and this corresponds to 6.0–13 mg kg^{-1} if taken orally (Benowitz and Henningfield, 1994; Woolf et al., 1997; Mayer, 2013). Further, Benowitz et al. (2009) indicated that the liver (a primary metabolite) using the CYP2A6 enzyme converts 70–80% of the nicotine in the human body into cotinine, which has a longer half-life of 16–20 h relative to 2 h for nicotine. Therefore, the observed amount of nicotine in maize grain is very low and considered negligible to cause a fatal effect to human when consumed as food. Our study recommends that further studies be conducted to evaluate the effect of maize planted as a subsequent crop after tobacco by detecting nicotine levels using a High-Performance Liquid Chromatography (HPLC). The HPLC can determine the nicotine levels in the pure state compared to the spectrophotometric method used in our study at 602 nm absorbance that could have detected the residue chlorophyll degradation as nicotine.

5. Conclusion

Soils are capable of retaining nicotine released by tobacco plants. The subsequent maize crop takes up the released nicotine and essential nutrients in soils, and their uptakes by the next crop in the cropping season also differed. Maize grain yield decreased slightly by 0.08 t ha^{-1} due to the reduced levels of P and K in soils previously cultivated with tobacco. Hence supplementation of P and K nutrients is essential in improving the yield of maize planted as a subsequent crop to tobacco. Maize roots took up nicotine with an average of 6.72 g plant^{-1}, and its concentrations decreased towards the leaves (0.060 g plant^{-1}). Thus, nicotine in maize grain was extremely low (0.011–0.016 g plant^{-1}) and considered negligible with no health effects to human upon consumption.

Declaration of Competing Interest

The authors declare to have no conflict of interests regarding this paper publication.

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