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Lipophilic Wood Extractives' Contamination of Water Bodies in the Vicinity of Pulp and Paper Mill, Southern Tanzania

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Abstract
This paper reports on the levels of fatty acids and selected sterols in effluents and nearby water bodies at Mufindi Paper Mill (MPM), southern Tanzania. Solid Phase Extraction (SPE) was used for sample extraction, and analysis was performed using Gas Chromatography-Mass Spectrometer (GC-MS). Fatty acids ranging from C11:0 to C25:0 were detected, with saturated fatty acids (SFA) being more abundant than unsaturated fatty acids (USFA). As for selected sterols, *β*-sitosterol and stigmastanol were more abundant than campesterol. The mean levels of SFA, USFA, β-sitosterol, stigmastanol, and campesterol (µg/L) were 538.28, 125.94, 1065.44, 1178.01, and 66.76, respectively, for untreated effluents, and 338.10, 139.03, 933.40, 153.92 and 57.82, respectively, for treated effluents. It was further established that the mean levels of SFA, USFA, β-sitosterol, stigmastanol, and campesterol (µg/L) were 321.29, 57.35, 58.37, 50.76, and 49.08, respectively, for effluents at the discharge point and 20.58, 17.72, 8.25, 10.55, and 6.05, respectively, at receiving water. Water bodies are therefore contaminated with lipophilic wood extractives since the mean concentration levels of USFA and sterols were above the lowest concentrations suspected to adversely affect fish (toxic limits (µg/L): USFA (2-8) and sterols (≥10)).

Keywords: Pulp and paper, wood extractives, fatty acids, sterols, contamination.

Introduction

The pulp and paper mill industries, which produce paper, pulp, and other cellulose-based products using wood as the main raw materials (Hewitt et al. 2007), have been noted as major sources of water pollution (Zhang et al. 2012). In pulp and paper production, a huge amount of freshwater (60 m^3 per ton of paper) is used (Buyukkamaci and Koken 2010), thereby generating a large amount of wastewater laden with high levels of organic and inorganic substances (Thompson et al. 2001, Ashrafi et al. 2015).

The pulping process can be carried out by either semi-chemical, chemical, or mechanical processes, in which the wood extractives are liberated and discharged into effluents (Pakkanen and Alen 2012). It has been observed that 85% of the water discharged from pulp and paper industries as effluents contain toxic contaminants (Patel et al. 2017). The effluents generated at the pulping stage contain lipophilic wood extractives, which possess a blend of compounds including resin acids, sterols, long chain aliphatic acids, alcohols, wax,

glycerides, sterol ester, hydrocarbons, steroid hydrocarbons, and ketones (Freire et al. 2003). Generally, unsaturated and saturated long-chain aliphatic fatty acids and sterols are highly toxic to aquatic organisms including fish (Gutiérrez et al. 1999).

Fatty acids have been reported as highly toxic when discharged into the environment, particularly to aquatic life on both flora and fauna (Wu et al. 2006). Fatty acids accumulate in fish tissues leading to chronic sublethal toxicity and genotoxicity in fish. Fatty acids also inhibit the functions of methanogenic bacteria, which play an important role in biological wastewater treatment (Ali and Sreekrishnan 2001). The toxicity of fatty acids varies, with polyunsaturated fatty acids reported as the most toxic to fish (Kilulya et al. 2012a). Polyunsaturated fatty acids in the environment undergo oxidation, forming oxylipins as the oxidative products, which are cytotoxic compounds for finfish and bacteria (Parrish 2013). In addition, peroxides of polyunsaturated fatty acids and superoxide anions formed after the oxidation process tend to destroy the gill cells of finfish and other aquatic organisms. Similarly, unsaturated fatty acids inhibit the functioning of gills in fish and cause death when the tolerable level is exceeded. The medium lethal concentration (LC_{50}) of unsaturated fatty acids in salmon or rainbow trout has been reported to range from 2.0 μgL^{-1} to 8.0 μgL^{-1} (Rigol et al. 2003).

Sterols reduce the sex steroid hormone levels in both sexes of fish and function as endocrine disruptors, leading to growth retardation and a decrease in gonad size (Van den Heuvel and Ellis 2002). This biological dynamic has been observed in rainbow trout, in which the β-sitosterols bind to the estrogen receptor, leading to the generation of vitellogenin in the liver of immature rainbow trout (Orrego et al. 2011). This causes the levels of testosterone, pregnenolone, and cholesterol in plasma to decrease (Mahmood-khan and Hall 2008). Various studies, including Kostamo et al. (2004), have reported that sterols become very toxic and even destroy the growth of fish larvae at concentrations of 10 μgL^{-1} and above. They also mimic the hormonal activities produced naturally by aquatic organisms due to the similarity in stereochemical structure with 17-β-estradiol hormone. The toxicity response of sterols to various fish including goldfish, zebrafish, and rainbow trout, has been expressed by the elevation of enzyme (Mixed-Function Oxygenase) activity indicating that sterols are highly toxic to aquatic organisms (Orrego et al. 2010).

However, despite their adverse effects on flora and fauna, wood extractives from the pulp and paper industry have not been investigated in the vicinity of Mufindi Paper Mills (MPM). Previous studies by Bernard et al. (2017, 2019) on paper mill effluents at MPM focused only on the levels and removal of chemical oxygen demand (COD), biological oxygen demand (BOD), total dissolved solids (TDS), total suspended solids (TSS), total solids (TS), turbidity and heavy metals. Therefore, this work reports on the identities, levels, and variations of fatty acids and sterols in the MPM wastewater and surrounding water bodies.

Materials and Methods

Study area and sample collection

The Mufindi Pulp and Paper Industry (Mufindi Paper Mills Limited) is located at Mgololo township, Mufindi District in Iringa Region, southern Tanzania. The Mufindi Paper Mills Ltd (MPM) is positioned at about 08° 55′S and 33° 32′E and raised at an altitude of about 1600 m above sea level.

Sampling was done at six sites, where the grab representative samples were taken as follows: Samples of effluents before treatment/clarification (SEB), samples of effluents after treatment/aeration (SEA), samples of effluents at the discharge point/stabilization (SED), samples of effluents at 2 km from the discharge point (SE2KD) taken from a stream of discharged effluent, samples at receiving water (WSRW), which is about 8 km from the discharge point, and samples at 1 km from receiving water (WS1KRW) taken at the middle part of the river and some at the sides

of the river, i.e. 9 km from the discharge point. Ten representative samples (1000 mL) were collected from every sampling site, i.e. SEB, SEA, SED, SE2KD, WSRW, and WS1KRW. Sixty (60) samples were collected, packed in glass bottles with a screw cap, iced, and transported to the University of Dar es Salaam Chemistry Department laboratories. Prior to sample extraction, samples were kept in the refrigerator at 4 °C.

Chemical reagents and solvents

The reagents and chemicals used in this work were of high purity and analytical grade. Hydrochloric acid (assay 37% w/v), acetone (assay 99.98% pure), methanol (assay 99.8% pure), anhydrous sodium sulphate (assay 99% pure), and n-hexane (assay 85% pure) were purchased from Fisher Scientific UK. Methyl tert-butyl ether (MTBE, Supelco) was purchased from Sigma-Aldrich. Other chemicals used as standard reference materials and prepared as 1000 μgL^{-1} stock solutions were: β-sitosterol (≥70% pure sigma grade), stigmastanol (≥80% pure), and cholesterol (≥99% sigma grade). FAMEQ-005 (Supelco 37 component FAME mix) contained different concentrations, i.e. 200 µg/mL, 400 µg/mL, and 600 µg/mL. Hexadecanoic acid and cholesterol were used as internal standards in fatty acids and sterols determination, respectively.

Sample preparation and extraction

Sample extraction was done by using the SPE method, in which octadecyl (C-18) was mainly used as a non-polar adsorbent (stationary phase) packed in the SPE cartridges. SPE cartridges were conditioned with methanol/water, loaded with the sample, and eluted by methyl tert butyl ether and n-hexane in 1:1% v/v. The eluted samples were derivatized to increase the stability, volatility and enhance the identification of the fatty acids suitable for GC-MS analysis. The extracted samples were mixed with 3 M methanolic HCl and heated in a water bath for 1 hour at 60 °C followed by cooling. Thereafter, extraction was done by using n-hexane for fatty acids and methyl tert butyl ether (MTBE) for sterols.

Instrumental analysis, identification and quantification

The analysis was performed using a Gas Chromatography coupled with Mass Spectrometer (Shimadzu GC-MS QP2010 Ultra). Ultra-pure helium (99.99%) carrier gas was passed through a separating column (Rtx-5MS, 30 m long × 0.25 mm i.d, 0.25 µm film thickness) at a flow rate of 1.0 mL/min. The oven temperature was initially set at 60 ℃, held for 2 min, and then increased to 320 ℃ at a rate of 15 ℃/min held for 10 min at a linear velocity of 36.4 cm/s. Split injection mode was used with 1 µL injection volume at 250 ℃ injection port temperature. The mode of ionization of the mass spectrometer used was electron impact mode set at 0.2 volts. The GC interface temperature was maintained at 300 ℃ and the ion source temperature at 230 ℃. The identification of sterols and fatty acid was performed by comparing the retention times and the mass spectra with those recorded in the GC-MS library (i.e., NIST 2011 libraries), based on mass fragmentation patterns and comparison with reference standards that were run under the same conditions as the sample analytes.

All fatty acids were quantified as fatty acid methyl esters (FAMEs) because the computed conversion factor (CF) of each fatty acid was nearly equal to unity, and thus there was no notable difference between the concentration of FAME and the corresponding fatty acid. The conversion factor (CF) for every fatty acid was obtained as the ratio of molecular mass of the respective fatty acid to that of fatty acid methyl ester (FAME) as shown in Equation 1.

$$CF = \frac{\text{Molecular mass of fatty acid (FA)}}{\text{Molecular mass of fatty acid methyl ester (FAME)}} \quad \text{.....(1)}$$

The concentration in each sample (µg/mL) was computed using Equation 2 stated by Ismailov (2013) with some modifications. It was multiplied by the dilution factor (DF) to obtain the actual concentration contained in a sample before its dilution, as indicated in Equation 2.

$$C_A = \frac{P_A \times C_S}{P_S \times PF} \times DF \quad \text{.....(2)}$$

Where: C_A = Concentration of analyte in a given sample (µg/mL); P_A = Peak area of analyte in a given sample (response); C_S = Concentration of the analyte in standard solution (µg/mL); P_S = Peak area of the analyte in standard solution (response); DF = Dilution factor; and PF = Preconcentration Factor (1000 since the 1000 mL sample was reduced to 1 mL after SPE).

Quality assurance

Reagent blanks chromatograms gave no significant peaks indicating that the concentration of analytes in reagent blanks were below the detection limit. The percentage recovery and precision obtained by using standard fatty acids and sterols spiked in distilled water are summarised in Table 1. The results show that accuracy and precision are within acceptable limits.

Table 1: Percentage Recoveries (n = 2) for fatty acids and sterols standards

Compound	Spiked amounts (mg/L)	Measured amounts (mg/L)	Recovery	Precision/RSD
C12:0	100	86.94 ± 1.29	86.94%	1.48%
C16:0	100	93.99 ± 5.07	93.99%	5.39%
C20:0	100	91.53 ± 3.79	91.53%	4.14%
β-Sitosterol	100	89.35 ± 4.18	89.35%	4.68%
Stigmastanol	100	86.28 ± 1.90	86.28%	2.20%

Note: C12:0, C16:0 and C20:0 were n-dodecanoic acid, n-hexadecanoic acid and n-eicosanoic acid, respectively.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 20 was used for statistical data analysis. The spatial variation of concentration of fatty acids and sterols in terms of mean value were compared with respective sampling sites by using one-way Analysis of Variance (ANOVA) with Tukey post-test. The significant variation of fatty acids and sterols (with confidence level set at $p = 0.05$) was assessed in industrial effluents compared with those of water bodies.

Results and Discussion

Fatty acids and sterols in paper mill effluents

Twenty (20) fatty acids (Table 2) were detected in both pulp and paper mill effluents and water bodies as fatty acid methyl esters (FAMEs). Among the fatty acids detected, twelve were saturated fatty acids (SFA) and eight were unsaturated fatty acids (USFA). The most abundant saturated fatty acids in both paper mill effluents and water bodies were heptadecanoic acid (C17:0), octadecanoic acid (C18:0), dodecanoic acid (C12:0), pentadecanoic acid (C15:0), docosanoic acid (C22:0) and pentadecanoic acid (C25:0) as shown in Table 2. Similarly, the most abundant unsaturated fatty acids (USFA) were 9-tetradecenoic acid, 9-hexadecenoic acid, 10-heptadecenoic acid, 11-eicosenoic acid, 9-octadecenoic acid, 9,12-octadecadienoic acid and 9,12,15-octadecatrienoic acid. On the other hand, three sterols were detected in both Mufindi Paper Mill (MPM) effluents and water bodies as shown in Table 2.

The results (Table 2) show that saturated fatty acids were more abundant compared to unsaturated fatty acids. A similar trend was also reported by Kostamo et al. (2004), who found that the concentration of saturated acids in pulp and paper mill effluents was

higher than that of unsaturated fatty acids. In an assessment of lipophilic wood extractives in pulp and paper mill effluents, Kilulya et al. (2012b) reported that saturated fatty acids are more abundant than unsaturated fatty acids, particularly when the eucalyptus trees are used in a pulping process. The reduced level of unsaturated fatty acids could be attributed to oxidation/epoxidation during the pulping process which forms oxylipins products, while saturated fatty acids remain unaffected (Parrish 2013). The results (Table 2) show contamination of water bodies since the levels of unsaturated fatty acids exceeded the range of 2.0 µg/L to 8.0 µg/L, which is suspected to cause toxicity to aquatic organisms (Rigol et al. 2003).

Sterols in untreated effluents (Table 2) show the trend: stigmastanol > β- sitosterol > campesterol. The levels of sterols obtained in this work were lower than those reported by Cook et al. (1997) and Van den Heuvel and Ellis (2002), in which levels ranged from 0.3 mg/L to 3.4 mg/L. It is worth noting that the levels of sterols in untreated effluents depend on the nature of raw materials and trees used in the pulping process (Kilulya et al. 2012b).

The effectiveness of the treatment process on the levels of fatty acids and sterols is shown in Figure 1. Results indicate that untreated effluents contained a high concentration of saturated fatty acids, which decreased to approximately 37% after treatment and stabilization and finally to 40% at the discharge point. Despite the decrease in concentrations of saturated fatty acids in untreated, treated and stabilized effluents, results show that fatty acids are resistant to decomposition, particularly when aeration treatment method is employed. Similarly, the levels of unsaturated fatty acids in effluent before treatment, after treatment (increased by ≈10%), and in the stabilization pond (decreased by ≈54%) show persistence in an aquatic environment. The levels of unsaturated fatty acids in the three stages (before/ after treatment and aeration) shows reasonable stability in aquatic environment. A slight increase in concentrations of unsaturated fatty acids may be due to the decomposition of more organic matter in the aeration pond during treatment. Despite a significant decrease in unsaturated acids during aeration, high levels of unsaturated acids are discharged to the environment. The persistence of unsaturated fatty acids may be attributed to their ability to undergo oxidation during the aeration process to form epoxide. The epoxides formed are more polar than the unsaturated fatty acids and hence reduce the possibility of being adsorbed on non-polar organic matter. In addition, the formation of epoxide affects bacteria responsible for the biodegradation of fatty acids in the treatment pond (Gentien and Bodennec 1998).

Table 2: Mean concentrations (μgL^{-1}) of fatty acids and sterols (n = 3) in Mufindi Paper Mill effluents and water bodies

Fatty acids and sterols	Untreated effluents	Treated effluents	Stabilized effluents/Discharge point	Discharged effluents after 2 km discharge point	Effluents at receiving water (8 km from discharge point)	Effluents 1 km away from receiving water
SFA						
C11:0	281.82 ± 151.79	185.39 ± 55.77	205.09 ± 90.61	100.75 ± 65.04	28.24 ± 11.98	13.08 ± 3.38
C12:0	548.81 ± 80.82	134.85 ± 12.25	362.00 ± 125.09	322.78 ± 27.45	26.25 ± 8.82	1.46 ± 0.26
C14:0	708.97 ± 350.44	17.02 ± 5.52	BDL	BDL	BDL	3.04 ± 0.14
C15:0	946.31 ± 203.51	91.62 ± 0.10	737.48 ± 179.29	592.58 ± 82.11	93.41 ± 29.02	7.05 ± 3.96
C16:0	65.69 ± 20.34	187.09 ± 44.09	25.19 ± 7.11	17.99 ± 5.58	7.77 ± 1.83	1.59 ± 0.46
C17:0	1318.13 ± 228.98	26.46 ± 1.99	1049.15 ± 235.35	854.11 ± 63.87	15.52 ± 5.01	3.30 ± 0.15
C18:0	138.65 ± 50.56	918.16 ± 153.55	42.27 ± 26.94	5.86 ± 2.29	6.23 ± 2.63	16.02 ± 2.90
C21:0	354.74 ± 122.28	1044.95 ± 72.87	90.79 ± 10.64	59.71 ± 20.49	14.78 ± 11.86	BDL
C22:0	666.34 ± 148.86	85.83 ± 11.68	534.87 ± 152.62	392.46 ± 66.62	20.32 ± 16.75	BDL
C23:0	974.49 ± 75.73	923.55 ± 75.24	2.49 ± 0.83	1.90 ± 0.40	1.30 ± 0.75	11.78 ± 4.04
C24:0	15.20 ± 2.19	83.00 ± 7.25	0.93 ± 0.02	1.61 ± 1.08	1.25 ± 0.30	1.42 ± 0.10
C25:0	440.23 ± 216.90	359.28 ± 115.44	483.95 ± 164.74	44.95 ± 21.86	11.31 ± 5.78	3.31 ± 1.23
Mean	**538.28 ± 48.52**	**338.10 ± 22.79**	**321.29 ± 33.61**	**217.70 ± 12.13**	**20.58 ± 3.29**	**6.21 ± 0.61**
USFA						
C14:1	48.05 ± 6.48	53.35 ± 11.68	55.93 ± 17.21	48.04 ± 10.44	27.18 ± 10.94	23.97 ± 2.15
C16:1	20.93 ± 10.44	77.78 ± 4.79	7.56 ± 3.26	3.37 ± 1.78	2.10 ± 0.74	BDL
C17:1	27.49 ± 0.10	43.90 ± 0.10	10.01 ± 3.14	8.20 ± 5.74	6.12 ± 1.42	BDL
C18:1	189.70 ± 90.03	266.90 ± 115.83	83.83 ± 18.09	64.64 ± 34.35	24.83 ± 13.46	7.25 ± 3.79
C18:2	487.39 ± 338.50	341.13 ± 255.22	137.29 ± 39.81	114.73 ± 57.48	22.70 ± 12.09	3.77 ± 0.46
C18:3	75.99 ± 12.44	70.16 ± 8.89	49.46 ± 8.00	49.82 ± 18.31	14.14 ± 7.44	4.21 ± 0.18
C20:1	32.03 ± 12.38	206.40 ± 68.29	BDL	14.54 ± 0.10	12.26 ± 3.55	5.87 ± 2.28
C22:1	BDL	52.58 ± 15.63	BDL	BDL	32.42 ± 0.10	23.49 ± 0.56
Mean	**125.94 ± 45.60**	**139.03 ± 36.16**	**57.35 ± 5.99**	**43.33 ± 8.81**	**17.72 ± 2.84**	**11.43 ± 0.62**
Sterols						
β- Sitosterol	1065.44 ± 209.31	933.40 ± 404.63	58.37 ± 17.35	47.81 ± 9.74	8.25 ± 1.89	5.76 ± 1.47
Stigmastanol	1178.01 ± 291.14	153.92 ± 26.59	50.76 ± 23.89	55.17 ± 10.76	10.55 ± 8.19	2.40 ± 1.06
Campesterol	66.76 ± 35.66	57.82 ± 26.17	49.08 ± 41.29	23.30 ± 20.66	6.05 ± 2.93	1.83 ± 0.66

Although β-sitosterol and stigmastanol occur at comparable concentrations in untreated effluents, β-sitosterol undergoes a smaller decrease after treatment than stigmastanol because it is not hydrolysed under alkaline conditions (Gutiérrez et al. 2001) during the pulping process. Thus, its concentration remains approximately constant. However, it undergoes a significant decrease before discharge since it remains as steryl esters in cooking liquor, causing it to have a high affinity for organic matter, thereby decreasing its concentration (Freire et al. 2003). It is worth noting that the low concentration of campesterol remains roughly constant until the discharge point. Thus, at the point of discharge, effluents collectively contain excessive concentrations of sterols above the 10 μg/L, regarded as capable of causing negative effects on fish (Kostamo et al. 2004).

The levels of the wood extractives studied in the surrounding water bodies are illustrated in Figure 2. It is worth noting that levels of fatty acids and sterols in water bodies follow a similar trend in untreated effluents, i.e. concentration of saturated fatty acids > unsaturated acids > sterols. The levels of fatty acids and sterols in water bodies were notably lower than in paper mill effluents at the discharge point. Saturated fatty acids decreased from 217.7 μg/L to 6.2 μg/L, while unsaturated fatty acids decreased from 43.3 μg/L to 11.4 μg/L at 2 km and 9 km from the discharge point, respectively. Similarly, β-sitosterol, stigmastanol, and campesterol decreased from 47.8 μg/L, 55.1 μg/L, and 23.3 μg/L to 5.8 μg/L, 2.4 μg/L, and 1.83 μg/L at the same respective points. The decrease in levels of fatty acids and sterols in water bodies might be due to their high affinity for organic matter and suspended matter sediments, which results in increased adsorption (Merilainen et al. 2006). Furthermore, the decrease in concentrations of both fatty acids and sterols in water bodies may be due to dilution of effluents. In addition, physical interaction with organic and inorganic matter available in the environment may have contributed to a decrease in some fatty acids and sterols as effluents flow from the stabilization pond to the environment. It is worth noting that at the last point of measurement (1 km after receiving water), the concentrations of unsaturated fatty acids and sterols collectively exceed the 8 μg/L and 10 μg/L thresholds for negative effects on fish.

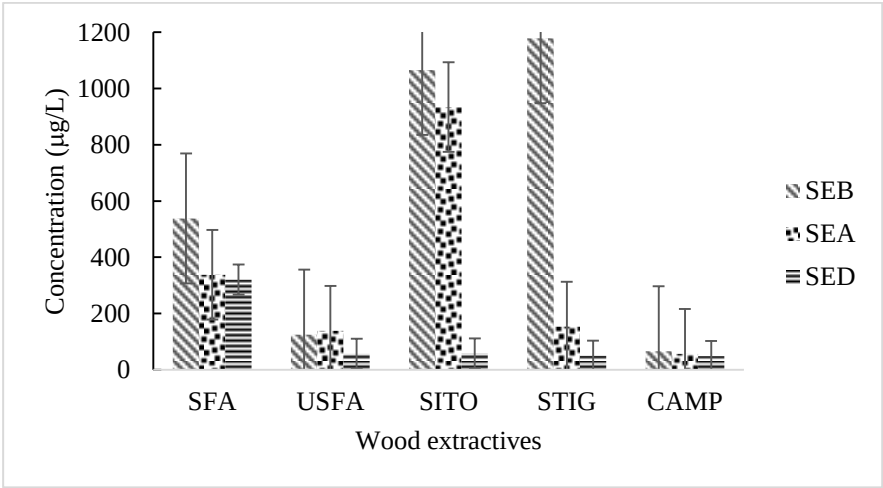

Figure 1: Levels of fatty acids and selected sterols in untreated effluents (SEB), treated effluents (SEA), and at the discharge point (SED)/stabilization pond. SITO = Sitosterol; STIG = Stigmastanol; CAMP = Campesterol.

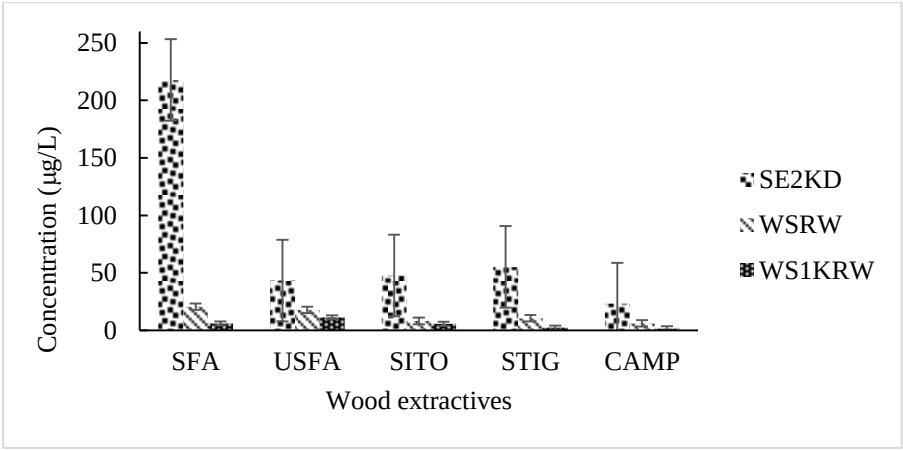

Figure 2: Levels of fatty acids and selected sterols in effluents at 2 km (SE2KD), 8 km (WSRW), and 9 km (WS1KRW) from the discharge point.

Spatial distribution of wood extractives

Figure 3 shows spatial variations of saturated fatty acids, unsaturated fatty acids, and sterols studied in this work from the paper mill effluents to the receiving water. The distribution of saturated fatty acids in effluents initially showed a small decrease from the processing point (untreated effluents) to the point of discharge (at 1 km). This may be attributed to the fact that saturated fatty acids are resistant to decomposition during the treatment process, as reported by Kostamo et al. (2004) and Karrasch et al. (2006). Saturated fatty acids showed a steady decrease in concentrations from 3 km (2 km from the discharge point) to 10 km (8 km from the discharge point), which is consistent with the observed trend that concentrations of lipophilic extractives decrease in the environment with distance.

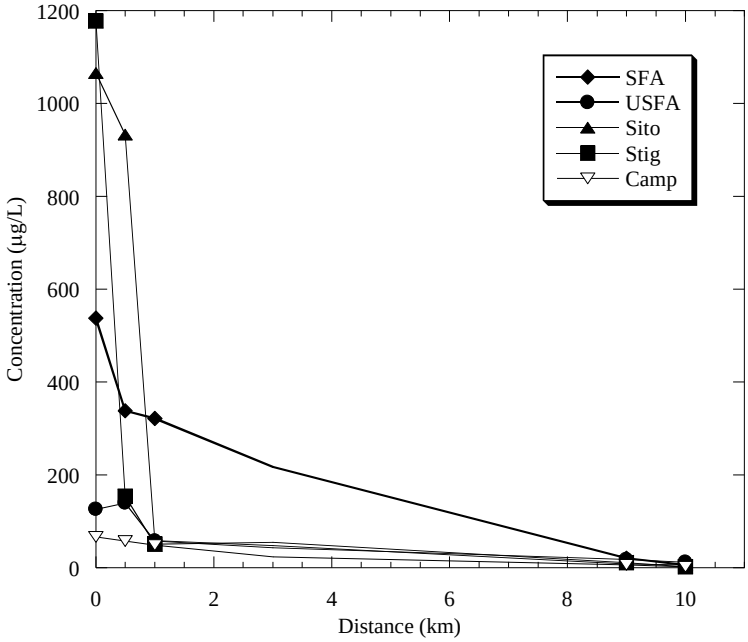

Figure 3: Variations in the levels of fatty acids and selected sterols in the vicinity of Mufindi Paper Mill (MPM).

For unsaturated fatty acids (USFA), a slight increase in concentrations from the processing point to a distance of 0.5 km was observed, probably due to the bio-decomposition of organic matters in the treatment pond, which caused an increase in concentrations of unsaturated fatty including 9-octadecenoic acid, 9,12-octadecadienoic acid and 9,12,15-octadecatrienoic acid. Unsaturated fatty acids showed a slight decrease after aeration and before the discharge point (1 km). Beyond 3 km (i.e. 2 km after the discharge point), the concentrations of unsaturated fatty acids remain largely constant, showing that the acids remain unchanged in the environment. Thus, water bodies are contaminated with unsaturated fatty acids since the levels of USFA at all points sampled including the final point at 10 km, exceeded the concentration range (2–8 μg/L), which is suspected to cause adverse effects on aquatic organisms.

As far as sterols are concerned, β-sitosterol showed a minor decrease in concentrations from the processing point (untreated effluents) to a distance of 0.5 km (at the treatment pond). This small variation in concentrations between treated and untreated paper and pulp mill effluents was also reported by Mahmood-Khan and Hall (2012) and Mahmood-Khan et al. (2015). However, a significant decrease in β-sitosterol level was observed as the distance increased to 1 km (at the discharge point). This decrease in the concentration of β-sitosterol in effluents might have been influenced by adsorption on organic matter and sediments (Gutiérrez et al. 2009) as well as dilution. Beyond the discharge point, the concentrations of β-sitosterol decrease slightly to 5.8 μg/L at 10 km (1 km from the receiving water). Stigmastanol showed a significant decrease in concentrations at 0.5 km (at the treatment pond), indicating that aeration treatment is more effective in removing stigmastanol (saturated sterols) than other unsaturated sterols. Beyond the point of discharge (1 km from the processing point), the concentrations of the stigmastanol remain nearly constant at 2.4 μg/L to a distance of 10 km, the last sampling point (9 km from the discharge point). Similarly, campesterol, which had the lowest concentrations compared to other sterols analysed, showed a slight decrease in concentrations with distance. Campesterol showed a slight, steady decrease in concentration from the processing point to a distance of 1 km at the discharge point. The same trend was observed up to 2 km, beyond which a constant concentration of 1.8 μg/L was recorded (Figure 3). Collectively, sterols at the two sampling points (at 3 km and 9 km) exceed the threshold concentration of 10 μg/L, while concentrations at the last sampling point are comparable to the threshold concentration.

Except for saturated fatty acids (SFA), which showed a high spatial distribution with a marked steady decrease in concentrations from 3 km to 10 km, other selected lipophilic wood extractives showed a minor decrease in concentrations. The decrease in concentrations from the processing point to 10 km indicates that lipophilic wood extractives are unequally distributed in the environment as the distance increases. This might have been influenced by physical interactions with organic matter, sediments, and the dilution of effluents in water bodies. Overall, concentrations of lipophilic wood extractives studied, except saturated fatty acids, are not affected by post-processing treatments like aeration, carried out at the mill. Thus, wood extractives occur in water bodies and contaminate the aquatic environment, whether singly or collectively. The lipophilic wood extractives, therefore, pose an environmental threat and may adversely affect fish and other aquatic organisms.

Statistical analysis of levels of wood extractives in MPM effluents and water bodies

In this statistical analysis, the sampling site was used as a conditional factor, in which the mean concentrations of fatty acids and selected sterols were compared to assess whether or not there was a significant difference in Mufindi Pulp Mill (MPM)

effluents and water bodies at Ruaha River. The results revealed that there were spatial variations in mean concentrations of saturated fatty acids (SFAs) among the sampling sites. This is because there was a significant difference in mean concentrations for the six sampling sites, in which the *p* value was 0.002 ($F_{(5, 55)} = 4.351$, $p = 0.002$), except for the first three sampling sites, SEB, SEA, and SED, which showed no significant difference as the *p* values were 0.617, 0.495, and 0.220, respectively. This indicates that the mean concentrations of SFA in untreated and treated effluents were more or less similar. Similarly, the mean concentrations of unsaturated fatty acids (USFAs) showed no significant differences among the sampling sites, in which the *p* value was equal to 0.051 ($F_{(5, 18)} = 2.803$, $p = 0.051$).

The mean concentrations of β-sitosterol, stigmastanol, and campesterol among the sampling sites indicated significant differences as the *p* value was less than 0.001. The only exception was observed in the first two sampling sites, SEB and SEA, where no significant variations were observed as p values were 0.762, 0.613, and 0.431 for β-sitosterol, stigmastanol, and campesterol, respectively. This indicates that there were no significant variations of mean concentrations for β-sitosterol, stigmastanol, and campesterol between untreated (SEB) and treated effluents (SEA). From the aforementioned, the aerated treatment system used at Mufindi Paper Mill Industry is not efficient in the removal of sterols contained in pulp and paper mill effluents.

Conclusion

The levels of fatty acids and sterols in SEB, SEA, and SED in pulp and paper mill effluents generally showed minor variations. The aeration system used at the Mufindi Paper Mill Industry to treat wastewater was therefore not sufficiently efficient at removing the lipophilic wood extractives (fatty acids and sterols). Thus, the wood extractives studied show the potential to contaminate aquatic environments. Fatty acids and sterols are spatially distributed within sampling sites as their levels decrease with distance from the processing point (SEB) to the surrounding environment. The levels of lipophilic wood extractives released into the environment are sufficient to affect fish and possibly other aquatic flora and fauna in the water bodies. From the statistical treatment of experimental data, a new wastewater treatment system, such as a hybrid of aerobic and anaerobic techniques or biological treatment (fungal and enzymatic treatment), is recommended to improve the removal of lipophilic wood extractives. Because of the effects of wood extractives on aquatic fauna and flora, it is further recommended that permissible limits for lipophilic wood extractives be specified by the environmental regulatory authorities.

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Conflict of Interest

The authors have no competing financial or non-financial interests in this work.

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