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Original research article

Evaluation of Toxicity of Paper Mill Sludge to Honey Bees and Analysis of Volatile Organic Compounds

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Abstract Large amounts of sludge produced by paper mill industries represent one of the most serious environmental problems in the world. Recently, beekeepers living in the neighborhood of the paper mill in Hwasan County, Youngcheon city, GB, Korea, became alarmed that honey bee colonies were dying off suddenly across the neighborhood. A preliminary study was conducted to evaluate the toxicity (oral, fumigation, repellent) of recycled solid paper mill sludge (SPMS) and leachate paper mill sludge (LPMS) to honey bee workers under laboratory conditions, and to analyze the volatile organic compounds (VOC). The SPMS and LPMS were separately subjected to a liquid-liquid extraction (LLE) at three temperatures to extract VOC (highest VOC yields: 1.52% SPMS and 0.34% LPMS). A total of 70 chemicals were detected in the VOC of paper mill sludges, of which 49 and 21 volatile organic compounds from SPMS and LPMS, respectively. The SPMS was dominated by high degree presence of stanols (saturated sterols), such as cholestanol, cholestan-3-ol and also saturated hydrocarbons. However, LPMS was characterized by the absence of sterols. Both SPMS and LPMS showed an influence on the olfactory behavior of honey bee on Y-tube assay, with repulsion rates of 72 and 68%, respectively. Both SPMS and LPMS at concentration of 100 mg/mL caused higher honey bee oral mortality than the untreated controls at 48, 72, 96 and 120 hours after treatment (highest oral mortality at 120 hr: 85.74% (SPMS); 93.51 % (LPMS)). A similar pattern was observed when honey bees were tested to fumigant toxicity. Both SPMS and LPMS caused significant higher mortality than the untreated control 24 hour after the exposure (highest fumigation mortality at 120 hr: 69.4% (SPMS); 56.8% (LPMS)). These preliminary results indicated that paper mill sludge could be partly responsible for sudden death and disappearance of honey bees, especially in hot humid summer days. With climate change, the risk of environmental chemical exposure to honey bee would pose greater attention.

Keywords Hymenopteran, Olfactory response, Paper mill sludge, Volatile contamination, Fumigation toxicity, Avoidance, Sudden death, Climate change

INTRODUCTION

Globally, pulp and paper industries represent one of the largest consumer of fresh water and also the major source of pollution (Thompson *et al*., 2001; Sumathi and Hung, 2006). The paper mill industry in South Korea produces 11 million tons of paper, and emits more than 1.3 million tons of sludge annually, which, goes mostly to landfills or burning (64%), ocean dumping (17%) and recycling (17%) (Lee, 2012). Most preferred way of recycling is to mixing with other organic materials and turn into agricultural compost. Primary and secondary treatment of wastes derived from wood fiber, recycled paper products, and non-wood fibers in pulp and paper

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mill industry produce various types of sludges. The wastewaters generated from pulping and bleaching processes in paper mill industry is a complex mixture of organic substances, such as phenolic compounds, organic acids, sugars and inorganic substances derived from the chemical additives used during the processing (Suntio *et al*., 1988).

The disposal of paper mill sludge is a serious problem for pulp and paper industries. Current disposal practices employed in developed countries include landfilling, composting, incineration and land reclamation applications (Abdullah *et al*., 2015). Paper mill sludge employed to reclaim a coal mine in Ohio, and it had little impact on fish, frogs, algae, or vegetation in a drainage lake (McFadden *et al*., 1995). Several studies have successfully demonstrated the use of paper mill sludges to amend disturbed soils, fostering revegetation and reducing erosion (Kost *et al*., 1997; Fierro *et al*., 1999). Abdullah *et al*. (2015) demonstrated that recycled paper mill sludge has also a potential to use as a fertilizer in soil amendment and also in neutralization of soil acidity. Study by Carpenter and Fernandez (2000) revealed that soils prepared by mixing paper mill sludges with other residuals were found superior to natural topsoil. Composting primary sludges with animal manures and other waste products has improved the low nutrient content of primary sludges (Baziramakenga and Simard, 2001; Lalande *et al*., 2003). For example, a reduction of cellulose content of primary sludge by 50% when composted with cattle manure (Valente *et al*., 1987).

The disposal practices like landfills, composting and storage are fast becoming unsustainable due to the scarcity of land for landfill sites and growing environmental and health concerns of the suitability of sludge constituents (pathogens, heavy metals and organic pollutants) in agricultural applications (Feldkirchner *et al*., 2003). Still, the most predominant usage of recovered sludge is for agricultural use such as fertilizer

Pulp and paper mill wastewater discharges into aquatic environment before its adequate treatment which causes eutrophication in aquatic system and health hazards in human and animal (Singh *et al*., 2016). The toxicity of paper mill sludge was evident by many studies (Hale *et al*., 2012; Oleszczuk *et al*., 2013) that solid recycled by-product (SPMS) can contain dangerous organic contaminants (e.g. polycyclic aromatic hydrocarbons (PAH), dioxins and furans) and inorganic (heavy metals). The presence of contaminants, therefore, poses a health risk to human and animal.

Large amounts of paper mill sludge are being accumulated, causing serious problems to honey bee keepers living in the neighborhood of the paper mills in Hwasan County, Youngcheon city, GB, Korea. Large sudden death of honey bees were observed in the area. Therefore, the present study was initiated to analyze the volatile chemicals and toxicity (oral, fumigation and repellent) of paper mill sludges to honey bee (*Apis mellifera*) under laboratory conditions.

MATERIALS AND METHODS

Materials

Collection of processing paper mill sludge and leachates

The apiary which reported the sudden bee death is located 300 m from the paper mill sludge processing facility in Hwasan County, Youngcheon city, GB, Korea (Fig. 1). The beekeeper keeps honey bees in 3 different sites nearby, but only one apiary site was severely damaged, leaving most of the vacant 150 honey bee hives.

Fig. 1. Map of the sampling location (marked by A) in Hwasan County, Youngcheon city, GB, Korea for the paper mill sludges used in this study.

Based on the beekeeper, few days with damping humid hot days in Early August 2019, all adult bees were disappeared with only developing larvae and pupae remaining (Personal communication with animal quarantine inspector, 2019). One and half month after the event, two different sludge samples ((recycled solid paper mill sludge (SPMS) and leachate paper mill sludge (LPMS)) were collected from the site on September 16, 2019.

Recycled solid paper mill sludge (SPMS) was the final recycled by-product, and used as a fertilizer. In the present study, SPMS was collected from the field storage one month before distributing to the farmers. In the recycling process, SPMS produced at the plant was being composted by blending the paper mill sludge with pig manure then, after dewatering, dried and took the form of granules (SPMS). Leachate paper mill sludge (LPMS) was a thick liquid derived from SPMS at the paper mill waste water treatment plant (WWTP) pond.

Extraction of volatile organic compounds by liquid-liquid extraction technique

Individual paper mill sludge (5 g each) was suspended in distilled water (100 mL each at 15, 25, 40°C) and were poured into the separatory funnel. Hexane (50 mL) was added to it. The mixture was then shaken vigorously for 5 min and then allowed to stand for 30 min. Following this, the organic and aqueous layers were separated. The aqueous layer was then further extracted again by shaking with hexane (50 mL). Both hexane extracts were combined, and the residual water was removed from the extract by treating with an anhydrous sodium sulphate (5 g). The extract was filtered and transferred into a round-bottom flask, followed by evaporation. The extract was stored in a refrigerator for further examination.

Analysis of volatile organic compounds (VOC) by Gas Chromatography-Mass Spectrometry (GC-MS)

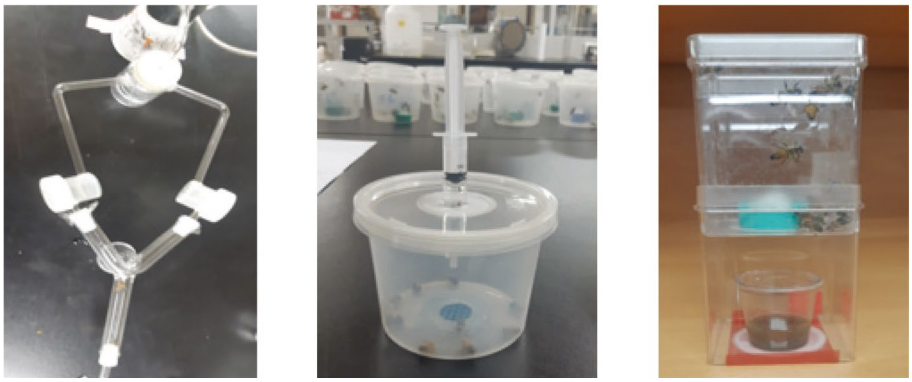
Hewlett-Packard HP 5977A Mass Spectrometer coupled with an HP-7890B GC was used to identify and quantify the VOC of SPMS and LPMS. Separation was performed on a non-polar column (HP-5MS-fused silica column; 5% phenyl methylpolysiloxane; 30 m×0.25 mm id; 0.25 μm film thickness, Agilent Technologies). VOC of SPMS and LPMS were diluted in acetone (1:100) prior to GC-MS analysis. Sample (1 μL) was injected in split mode with a 1:10 split ratio. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. The oven was initially set at 40°C for 3 min, then the temperature was programmed to increase by 6ºC/min until it reached to 150°C, the temperature was further programmed to increase by 10ºC/min until it reached a final temperature of 320ºC, which was kept for 3 min. The MS readings were scanned in the range of 40~500 amu in full-scan mode with electron impact ionization energy of 70 eV. The temperatures of the ion source and injector base were maintained at 230 and 270°C, respectively. Compounds were identified by comparing the mass spectrum of individual VOC with those listed in the MS Library Database.

Toxicological test

We have collected young adult workers from healthy honey bee (*Apis mellifera*) colonies in the experimental apiary of Andong National University. Honey bee colonies are all from the same breeding lines and requeened in the previous autumn season. The collected bees were released into the ventilated test cage made of stainless steel. In each cage 10 bees were apportioned. Then the bees were starved for 2 hr in the experimental room at 25±2°C and 50~70% relative humidity.

Olfactory response of honey bee

A Y-tube olfactometer system previously described by Li *et al*. (2014) was used to test the orientation responses of honey bees towards to odors emanating from SPMS and LPMS (50 mg/mL in 50% sugar solution, each). The Y-tube olfactometer (Fig. 2) comprised of a central tube and two lateral arms (each 8 cm long and 18 mm internal diameter). Each arm was connected to an odor chamber (cryogenic vial; 4.3 cm in height, 3.0 cm inner diameter; 20 mL) holding the test sample. Each odor chamber had inlets for the incoming air and outlets for odors to exit the Y-tube. A charcoal-filtered and humidified air stream was passed into each arm at a flow rate of 250 mL/min (DK-800 Air Pump) to allow experimental odors moved towards the decision-making area. A mesh screen was placed at each of the endpoints of the olfactometer to prevent honey bees escaped from the test area and from a direct contact with the test samples. Honey bees were starved for over 2 hr before being tested. The test samples were separately applied to pieces of cotton. A 10 μL aliquot of each sample in sugar solution was dripped



(A) (B) (C)

Fig. 2. Bioassay set-ups: (A): Y-tube set-up; (B): Oral toxicity set-up; (C): Fumigant toxicity set-up.

onto a piece of cotton that had been placed inside one arm, and another piece of cotton permeated with 10 μL of sugar solution was placed in the other arm of the Y-section as a control. To prevent any positional bias in the behavior of the honey bees, the relative position of the tested stimulus and its corresponding control were alternated between replicates. A clean Y-tube was used for each test in order to avoid carryover of odors. The Y-tube was illuminated with red light by means of an incandescent light bulb to preclude their use of visual cues during the experiments. One honey bee at a time was introduced into the Y-tube after the airflow had been initiated. It was assumed to have made a choice when the honey bee walked more than 2/3 length of the treated source or control arm and stayed there for approximately 1 min or when it frequently visited the arm. A "no choice" decision was recorded if the honey bee had not moved after 5 min. Each treated or control cotton was used only once and was then replaced with a fresh cotton for the next individual, and each individual honey bee was used only once in the experiment. 50 honey bees were used for individual test samples. Response rate (%) and repulsion rate (%) of SPMS and LPMS on honey bees in the Y-tube olfactometer was computed using a formula reported by Li *et al*. (2014).

$$\text{Response rate}\,(\%) = \frac{\text{Responding honey bees}}{\text{All honey bees tested}} \times 100$$

$$\text{Repulsion rate}\,(\%) = \frac{\text{Responding honey bees} - \text{honey bees showing attraction}}{\text{Responding honey bees}} \times 100$$

Acute Oral toxicity to honey bee

Test samples (100 mg/mL) of SPMS and LPMS were prepared in 50% sucrose solution. A plastic Kovax-Syringe (5 mL) with the open end narrowed to about 2 mm diameter was used as feeder unit (Fig. 2). An amount of 5 mL of prepared doses was taken into the feeder unit without any air bubbles. The feeder unit containing 50% sucrose solution alone was considered as control. The experiment was replicated thrice. Then the feeder volumes were taken and placed into the respective test cages. The honey bees were allowed to consume the dose for maximum of 6 hr and there after the feeder units were removed from the test cage and weighed to measure the dose consumed. The feeder unit containing sucrose solution alone was replaced into the test cage as soon as the dosage was removed. Then the bees are observed for the mortality on 3, 12, 24, 48, 72, 96 and 120 hours.

Fumigant toxicity to honey bee

SPMS and LPMS (2.9 g/L Air, for each) in a cap were placed at the bottom of a rectangular fumigation box (6.2 cm in length, 6.2 cm in width, 17 cm in height; 695 mL), with mesh-holes fitted approximately 7.5 cm above the bottom of the fumigation box (Fig. 2). Batches of 10 honey bees were introduced into each box by placing them in separate compartment containing food (50% sucrose solution), thereby preventing their direct contact with the test samples. Both ends of the box were sealed with using parafilm to prevent from leaking. The controls had no test sample. Honey bees were maintained at 23 ± 1°C and 30% relative humidity. Triplicates were set

up for each treatment. Mortality of adults was observed at 3, 12, 24, 48, 72, 96 and 120 hours. Honey bees were considered dead if they did not move.

Statistical analysis

Olfactory responses of honey bees to test samples were compared using the Chi-square test (SPSS Statistics, v. 16) with $p<0.05$ as an indicator of statistical significance. All toxicity assays were carried out in triplicate, and the results were presented as the mean mortality (%). Two-way ANOVA analysis, followed by Tukey multiple comparison tests, was used to compare test the effect of test samples on honey bees at each time point.

RESULTS AND DISCUSSION

Characterization of volatile organic compounds of the sludge

Recycled solid paper mill sludge (SPMS) and leachate paper mill sludge (LPMS) were subjected to a liquid-liquid extraction (LLE) technique at different temperatures to give volatile organic compounds (VOC), with the highest 1.52% VOC yield for SPMS at 40°C. The volatile yields vary according to the type of sample collected as indicated in Table 1 below.

A total of 70 chemicals were detected in the VOC of paper mill sludges, of which 49 (Table 2) and 21 (Table 3) volatile organic compounds from SPMS and LPMS, respectively. The SPMS was dominated by high degree presence of stanols (saturated sterols), such as cholestanol, stigmastanol, cholestan-3-ol and also saturated hydrocarbons. It was also noted that high concentration of stanols were also detected in livestock manure (Sheesley *et al*., 2013). However, LPMS was characterized by the absence of sterols. Octathiocane was found the major constituents of LPMS, accounting 36.27% of the total volatile. There were also significant amount of benzene derivatives and polycyclic aromatic hydrocarbons (PAHs) present in both SPMS and LPMS. The presence of such compounds in paper mill effluent was also evident in a study conducted by Lacorte *et al*. (2003). Wood extractives (resin and fatty acids, sterols, etc.), diterpene alcohols, and juvabiones were also reported to be present in various paper mill effluent streams (Leach and Thakore, 1977).

Table 1. Percentage yields of volatile organic compounds from two paper mill sludges (SPMS and LPMS)

Temperature (°C)	VOC Yield (%; w/w)	
	SPMS	LPMS
15	0.24	0.01
25	0.92	0.08
40	1.52	0.34

Olfactory responses of honey bees to paper mill volatile organic compounds

Y-tube olfactometers are conveniently small devices used for rapid screening of insect olfactory response to a choice between an odor and clean air or two different odors (Geier and Boeckh, 1999). When honey bees were exposed to a choice between 5% SPMS in sugar solution and sugar solution (control), the majority of honey bees tested walked quickly towards the far end of the control arm of the Y-tube. Interestingly, individual honey bee made a choice within the first minute period.

In fact, there was a significant difference in the repulsion rate of honey bee workers when they were presented with the odor of SPMS and sugar solution (control) ($\chi^2=13.863$, df = 5, $p=0.017$), with a response rate of 100% (zero no-choices were recorded in the experiments) and repulsion rate of 72% (Fig. 3). A similar pattern was observed when honey bees were exposed to a choice between LPMS and sugar solution ($\chi^2=13.863$, df = 3, $p=0.003$). When honey bees were exposed to SPMS and LPMS in Y-tube test, it had a low response rate of 74%. It was also noted that there was no significant difference in the repulsion rate of honey bee workers when they were presented with the odor of SPMS and LPMS ($\chi^2=2.634$, df = 2, $p=0.268$). However, arithmetically, honey bee workers' repulsion rate to SPMS tended to be higher compared to LPMS.

These results indicated that paper mill sludge, particularly SPMS, was repellent to the workers of honey bees, thus, VOC of both SPMS and LPMS could be partly the cause of disappearing bees.

Oral toxicity of paper sludge to honey bee

Caged bees fed in test samples (SPMS and LPMS, separately) at a concentration of 100 mg/mL remained

Table 2. Volatile organic compounds from recycled solid paper mill sludge (SPMS)

Compound number	RT (min)	Compound Name	Percent composition (%)
1	26.33	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	0.42
2	27.49	Butyl octyl ester-1,2-benzenedicarboxylic acid	1.45
3	27.56	7-Amino-7H-S-triazolo[5,1-c]-S-triazole-3-thiol	0.42
4	28.22	Octathiocane	8.80
5	28.56	2,3,4-trimethyl-benzo[h]quinoline	0.20
6	29.14	1-methyl-3-nitro-benzene	0.46
7	29.66	1,1'-sulfonylbis(4-methyl-)benzene	0.66
8	29.81	Tetratetracontane	0.29
9	30.68	Tricosane	0.44
10	31.51	Tetracosane	0.74
11	32.30	Hexacosane	1.20
12	32.65	Decyl octyl ester-1,2-benzenedicarboxylic acid	1.18
13	33.06	Heneicosane	1.31
14	33.49	3-Ethyl-tetracosane	0.66
15	33.80	9-Hexyl-heptadecane	1.35
16	34.12	Di-n-octyl phthalate	0.38
17	34.24	Eicosane	0.36
18	34.31	2,6-Dimethyl-2-trans-6-octadiene	0.46
19	34.50	9-Octyl-heptadecane	1.30
20	34.71	Squalene	0.65
21	35.19	Nonacosane	0.71
22	35.86	Octadecane	0.46
23	36.46	3β,5β-Cholestan-3-ol	17.98
24	36.56	2,3-Dihydro-2,8-dimethyl-Benz[b]-1,4-oxazepine-4(5H)-thione	0.30
25	36.72	Cholestan-3-one	13.15
26	36.77	Cholestanol	2.63
27	37.01	5α-Cholestan-3-one	2.87
28	37.13	3b,5α-3-methoxy-cholestane	1.53
29	37.39	1-methyl-2-phenyl-1H-Indole	2.04
30	37.44	2,4-Dimethyl-benzo[h]quinoline	0.35
31	37.51	Cholest-4-en-3-one	2.43
32	37.58	Di-n-decylsulfone	1.74
33	37.63	Palmitic acid vinyl ester	1.49
34	37.70	Stigmastanol	5.84
35	37.79	5-Methyl-2-phenylindolizine	0.91
36	37.95	Stigmastan-7-one	5.09
37	38.00	1H-Benzo[4,5]furo[3,2-f]indole	1.84
38	38.16	5-(1,3,5-trimethyl-4-pyrazolyl)amino-1,2,4-triazol-3-amine	1.30
39	38.24	1,4-Bis(trimethylsilyl)benzene	1.28
40	38.29	4-Methyl-2-trimethylsilyloxy-acetophenone	0.99
41	38.38	2-(1-Adamantyl)ethyl ester-phenylacetic acid	0.79
42	38.44	[4-(1,1-dimethylethyl)phenoxy]-, methyl ester Acetic acid	1.33
43	38.66	Hexamethyl-cyclotrisiloxane	0.64
44	38.75	N-Methyl-1-adamantaneacetamide	1.53
45	38.82	1,2-Bis(trimethylsilyl)benzene	1.70
46	39.05	2-Ethylacridine	0.61
47	39.13	Tris(tert-butyldimethylsilyloxy)arsane	0.35
48	39.23	Tris(trimethylsilyl) ester arsenous acid	1.00
49	40.20	1,2-Benzisothiazol-3-amine tbdms	0.40
		Total	96.36

active the first 12 hours period of the experiment. Indeed, no mortality was observed during this period of the experiment (Fig. 4). Routine worker behavior such as grooming appeared normal for bees across all tested groups, including the behaviors of bees fed in SPMS and LPMS. At the observation time of 24 hour, when dead bees were sampled, no differences were observed in comparison to control bees and bees fed with sugar solution treated with SPMS and LPMS ($p > 0.05$).

SPMS at concentration 100 mg/mL caused higher

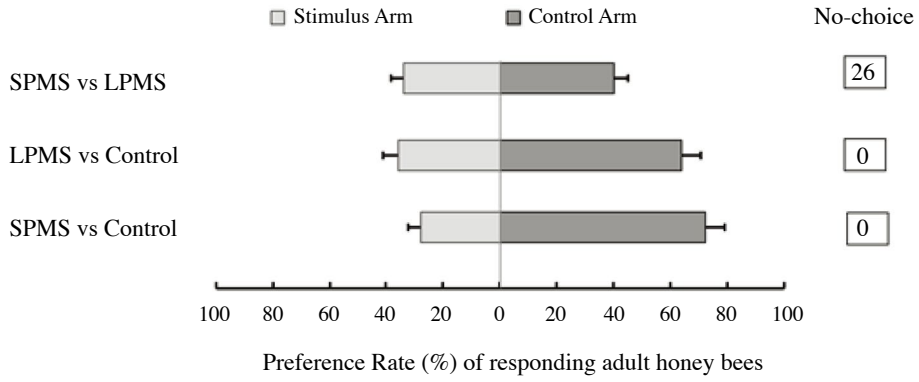


Fig. 3. Response of honey bees in Y-tube olfactometer in dual choices b/n the treatment (Test sample + sugar solution) and the control (sugar solution); N = 50 for all treatment. SPMS = Recycle solid paper mill sludge; LPMS = leachate paper mill sludge. Note: The same letter in the pair of test (in the raw) indicates no significance difference ($p > 0.05$), and different letters in the pair of test means there is a significance difference.

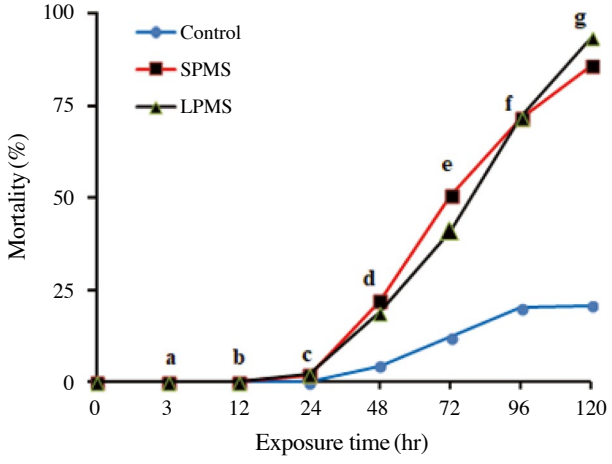


Fig. 4. Oral toxicity of two paper mill sludge (SPMS and LPMS) to honey bees, *Apis mellifera* workers. Sludge materials were mixed with 50% sugar solution for feed. (Note: Letters a, b, c refer to no significance difference among the three tested samples; d, e refer to significance difference among the three tested samples; f refers to there is a significance difference between treated samples (SPMS and LPMS) and the control, however, no significance difference between SPMS and LPMS; g refers to significance difference among the three tested samples). (No significance difference $p > 0.05$; Significance difference $p < 0.05$).

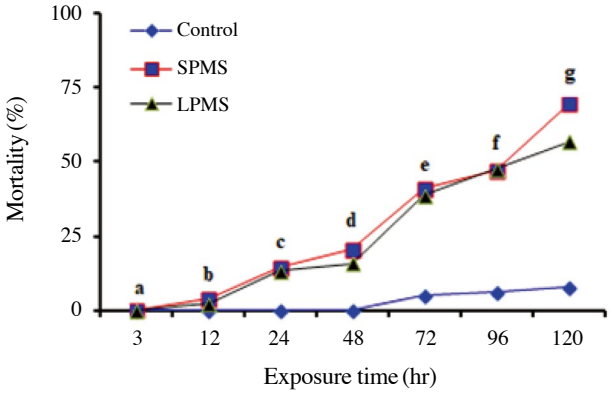


Fig. 5. Fumigant toxicity of two paper mill sludge (SPMS and LPMS) to honey bees, *Apis mellifera* workers (Note: Letters a & b refer to no significance difference among the three tested samples; c, d, e & f refer to there is a significance difference between treated samples (SPMS and LPMS) and the control, however, no significance difference between SPMS and LPMS; g refers to significance difference among the three tested samples). (No significance difference $p > 0.05$; Significance difference $p < 0.05$).

honey bees mortality than the untreated controls at 48, 72, 96 and 120 hours from test initiation in oral toxicity tests ($p < 0.05$) (Fig. 4). A similar pattern was observed when honey bees fed with LPMS. In fact, the highest percent oral toxicity mortalities were recorded at 120 hours after the oral toxicity initiation (LPMS = 93.51%; SPMS = 85.74%). Therefore, long persistent exposure of honey bees to paper mill sludge was more likely to put honey bees to a sudden and unexpected death. In a study conducted by Leach and Thakore (1977) indicated that 70~100% of toxicity in various paper mill effluent streams was due to the presence of wood extractives (resin and fatty acids, sterols), diterpene alcohols, and juvabiones.

Fumigant toxicity of paper sludge to honey bee

Fumigant toxicity of both SPMS and LPMS to honey bees were also investigated at concentration of 2.9 g/L Air. Both SPMS and LPMS were characterized by strong unpleasant odors. As indicated in Fig. 5, in the first 12 hours of the experiments, no significant differ-

Table 3. Volatile organic compounds from leachate paper mill sludge (LPMS)

Compound number	RT (min)	Compound Name	Percent composition (%)
1	26.46	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	0.58
2	27.49	Dibutyl phthalate	14.11
3	28.22	Octathiocane	36.27
4	28.89	Heptadecane	0.46
5	29.81	Docosane	1.00
6	30.68	Tricosane	1.80
7	31.51	Heneicosane	4.60
8	32.30	Hexacosane	7.36
9	32.65	Monobutyl ester-1,2-Benzenedicarboxylic acid	0.77
10	33.06	10-Methyl-eicosane	6.22
11	33.80	9-Octyl-heptadecane	6.09
12	34.50	Octacosane	5.56
13	35.18	Nonacosane	3.82
14	35.84	Tetracosane	3.29
15	36.43	2,4-Dimethyl-Benzo[h]quinoline	0.45
16	36.48	Eicosane	1.37
17	36.69	2-(1-Adamantyl)ethyl ester phenylacetic acid	1.38
18	37.10	9-Octyl-eicosane	0.80
19	37.50	5-(1,3,5-Trimethyl-4-pyrazolyl)amino-1,2,4-triazol-3-amine	0.09
20	37.57	1,2-Benzisothiazol-3-amine tbdms	0.16
21	37.70	5-Methyl-2-trimethylsilyloxy-acetophenone	0.36
		Total	96.49

ence were observed among the tested samples (SPMS, LPMS and untreated; $p>0.05$). 24 hours after exposure to the test samples, there were significant difference treated ample (SPMS and LPMS) and the control groups ($p<0.05$). The highest percent mortalities were recorded at 120 hours from test initiation in fumigant toxicity (LPMS = 56.8%; SPMS = 69.4%. SPMS and LPMS together can contaminate honey bee colonies through olfactory, oral and fumigant toxicities, and it puts honey bee at risk to jeopardize their numerous social interactions.

CONCLUSIONS

The one-time reported high loss rates of honey bee colonies in the vicinity of paper mill sludge processing facility could be attributed to diverse stressors. Indeed, the present study clearly showed that honey bees at the vicinity of the sludge processing facility are at risk due to oral and fumigation toxicities. Orientation behavioral of the honey bees is also at the risk due to the VOC odor emanating from the sludge. In fact, the toxicity of paper mill sludge to bees may be exacerbated due to a climate change as the warmer and humid days during summer is expected to increase and honeybee would expose more stressors.

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