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Occupational exposure and DNA strand breakage of workers in bottom ash recovery and fly ash treatment plants

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ABSTRACT

Various environmental hazards and metals are liberated either into bottom ash or carried away with gases and subsequently trapped in fly ash. Many studies have reported an increase of DNA damage is related to hazardous exposure of municipal waste incinerators. By detecting DNA damage, we compared the DNA migration imposed in workers potentially exposed to hazardous substances, including PCDD/Fs, metals, and silica particles, at a bottom ash recovery plant and fly ash treatment plants in Taiwan. Higher tail moment (TMOM) was found in workers at fly ash treatment plants (7.55) than in the workers in bottom ash plants (2.64), as well as those in blue collar was higher than in white collar workers (5.72 vs. 3.95). Meanwhile, the significantly higher DNA damage was also shown in workers with high integrated exposure score than those with low. The air samplings for particle mass, Cr, and Al concentrations also showed the higher levels in fly ash treatment plants than in the workers in bottom ash plants. Meanwhile, the air samplings inside the two plants suggested that the particle size might be important to affect the workers inhaling the metal into the human body and finally caused to their DNA damage. The data concluded that an elevated DNA damage may be expected in workers at fly ash treatment plants than those at bottom ash plants; however, the occupational hazards in both types of plants, especially at different particle size interval, need more thorough assessment in future studies.

1. Introduction

Various environmental hazards and metals are liberated either into bottom ash or carried away with gases and subsequently trapped in fly ash. The fly ash and ambient emissions of municipal solid waste incinerators are well known to contain polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), polycyclic aromatic hydrocarbons (PAHs), other organics, metals, and gases [1,2].

Study have identified an increasing DNA damage in related to the negative control *in vitro* study by treatment with the percolate bottom ash of municipal waste incinerator [3]. Hazardous substances such as PCDD/Fs, mercury and other silica particles cause the increase of oxidative damage, including malondialdehyde (MDA), 8-hydroxydeoxyguanosine (8-OH-dG), and DNA strand breakage in residents around industrial areas or workers in municipal waste incinerators [4–7]. In addition, DNA breakage (the tail moment of single cell gel electrophoresis by comet assay, TMOM) *in vitro* study, one of DNA damage, appeared to be associated with dioxin-like chemicals from waste incinerators [8]. Concluded the above findings, the toxic effect of residue from municipal waste incineration has been proved. The toxicity characteristic leaching procedure (TCLP) for bottom ash and fly ash have suggested that PAHs and some metal concentration, especially for Pb and Zn, in fly ash was higher than those of bottom ash [9–11]. It is highly like that the workers might overexpose to the hazards such as PCDD/Fs, PAHs and metal concentration while they handled the residue of incineration, especially in fly ash treatment plant.

In Taiwan, the bottom ash and fly ash were sent separately to the bottom ash recovery plant and fly ash treatment plants after incineration of the municipal waste. Few epidemiological studies have reported the oxidative stress in workers at municipal waste incinerators [6,12], little is known about the status of oxidative stress in workers at the kind of plants potential with much more PCDD/Fs, silica particles and metal exposure. Here, we compared the DNA strand breakage in workers potentially exposed to hazardous substances at a bottom ash recovery plant and fly ash treatment plants by using the alkaline version of comet assay to detect basal DNA damage.

Abbreviations: SCEG, Comet assay (single cell gel electrophoresis); TMOM, the tail moment; MDA, malondialdehyde; PCDD/Fs', polychlorinated dibenzo-*p*-dioxins and dibenzofurans.

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2. Materials and methods

2.1. Subjects

The study was conducted in a typical bottom ash recovery plant and three fly ash treatment plants. A bottom ash recovery plant handles the bottom ash from municipal waste incinerators, from which pieces of metal are recovered. The fly ash treatment plant solidifies fly ash by adding cement and chelator. We did a pre-sampling walk through to comprehend the layout of each work site and manufacturing processes in these plants. In principle, both types of plants were identified as emission sources of PCDD/F, metals, and silica particles because they handle the residues of waste incineration.

37 workers were recruited from a bottom ash recovery plant and 41 workers from fly ash treatment plants in Taiwan. The employees were assigned to blue collar or white collar groups based on their operational departments or job titles. Each employee was asked to fill out a questionnaire asking for information about personal characteristics (gender, age, height, weight, residence neighborhood, etc.), life style (e.g., tobacco usage, and alcohol intake), and occupational histories (e.g., working history at current place of employment, working environment, job titles, periods of employment, and use of protective equipment).

This study was approved by the Ethics Committee of the Kuang Tien General Hospital (Taichung, Taiwan).

2.2. Collecting blood

The sampling day was Friday, the last working day of the week. Friday was chosen as it would represent the highest accumulation of hazardous chemicals for the week in this type of environment. After they signed consent forms, which was approved by the Ethics Committee of the Kuang Tien General Hospital (Taichung, Taiwan) and they had completed an overnight fast, each participant provided 1 mL of venous blood, drawn into chemically clean tubes that contained heparin. After cryoprotectants (1:1 ratio) had been added, the blood was stored at −85 °C until the comet assay.

2.3. Blood comet assay

The comet assay protocol was referred from our previous report [13]. First, 150 μL of 1% normal-melting point agarose (NMA) was applied to the first layer of the slide. We then mixed 50 μL of whole blood with 250 μL of 1% low melting point agarose (LMA), and applied 130 μL of that mixture to the second layer of the slide. After applying another layer of 1% NMA (150 μL), we immersed the slides in cold lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 1% *N*-sodium lauroyl sarcosinate) for 1 h at 4 °C. The slides were then placed in an electrophoresis tank, allowing the DNA to unwind for 15 min in the alkaline solution (300 mM NaOH and 1 mM Na_2EDTA). Electrophoresis was then done at 300 mA for 20 min in the same alkaline solution at room temperature. Next, the slides were neutralized by adding 0.4 M Tris–HCl buffer (pH 7.5) and stained with ethidium bromide. The image of DNA strand breakage was visually analyzed using comet scores according to the method of Chia et al. [13]. The tail moment (TMOM) was determined using Comet Assay IV (Perceptive Instruments Ltd., Haverhill, Suffolk, UK) according to the formula:

$$\text{TMOM} = \text{TDNA(DNA in tail as a \% of total DNA)} \times \text{TDx(DNA tail length)}.$$

The breakage of DNA strands occurred due to oxidative damage and a comet was formed by electrophoresis. The longer the DNA tail length, presented the higher the percentage of breakage.

Table 1
Calculation of exposure index based on each exposure score.

Variables	Definition	Scores
Age	>35 years old	1
	≤35 years old	0
Exposure to ash	Yes	3
	No	0
Periods of employment[a]	>3.33 years	2
	≤3.33 years	0
Working time per day[a]	<4 h/day	1
	≥4 h/day	2
White collar/Blue collar	White collar	0
	Blue collar	2
Use of protective equipment	Yes	0
	No	1
Ever work in other factories	Yes	1
	No	0

[a] The median of the variables.

The tail moment is considered one of the best indices of comet formation obtained in computerized analysis [14].

2.4. Exposure index

The exposure index was used to calculate the actual exposure for workers in different exposure scenarios. Age, whether they were exposed to ash, working duration, working hour per day, white collar/blue collar, whether they use protective equipment, and whether they had worked in other similar plants were all included to calculate the exposure index. The scores of exposure index are given in Table 1.

2.5. Ambient samples

Air samples were taken inside the plants using an eight-stage cascade impactor (Personal Cascade Marple Impactor Model 225-50-001; SKC Inc., 84, PA) with 0.45 μm pores and 37 mm-diameter mixed cellulose ester filters (MCE, Nucleopore, Inc., Cabin John, MD). The aerodynamic diameter ranges were <0.4, 0.4–0.7, 0.7–1.0, 1.0–3.5, 3.5–6.5, 6.5–10, 10–15, 15–21 μm. Mucilage was sprayed on filters before sampling to avoid particle bounce. Pre- and post-air sampling, the filters were conditioned at the same temperature and humidity. In addition, the filters were weighed 48 h post-conditioning. The post-sampling weights were subtracted from the pre-sampling weights to provide the particle mass in this ambient sampling. Personal air pump samplers with a flow rate of approximately 2.0 L/min were used. We took five and 15 samples in operating departments of the bottom ash recovery plant and fly ash treatment plants, separately. The sampling time was about 8 h per sample.

2.6. Metal analysis by inductively coupled plasma-optical emission spectrometer (ICP-OES)

Particles were digested in a 1200 W microwave oven (Mars, microwave digestion system, CEM) according to Tsai et al. [15] to ensure accurate and reliable analysis of metals in the particles. The digested solution was a mixture of 8.0 mL 65% HNO_3 and 2.0 mL 30% H_2O_2. All reagents were prepared using chemicals supplied by Merck (Analytical grade). Inductively coupled plasma-optical emission spectrometer (ICP-OES, ICP-OES Optima 2100DV, PerkinElmer) was used to analyze the metal concentrations.

Table 2
The differences in DNA strand breakage levels (TMOM) between different age groups, smoking habit, alcohol-drinking habit, and other life styles (Wilcoxon Rank Sum Test).

Variables	*N*	TMOM		*p* value
		Mean ± standard deviation	Min–max	
Age (years)[a]				
35 or over	31	6.95 ± 8.17	1.78–37.53	0.190
Below 35	47	4.09 ± 2.38	1.59–12.87	
Smoking status				
No	39	4.94 ± 4.75	1.59–25.03	0.738
Yes	39	5.50 ± 6.39	1.78–37.53	
Alcohol-drinking				
No	50	4.94 ± 4.42	1.59–25.03	0.959
Yes	28	5.73 ± 7.32	1.80–37.53	
Use of betel nut				
No	67	5.25 ± 5.92	1.59–37.53	0.332
Yes	10	5.09 ± 3.30	1.78–12.87	
Excise regular				
No	45	5.12 ± 6.00	1.78–37.53	0.964
Yes	33	5.35 ± 5.10	1.59–25.03	

TMOM (Tail moment) = TDNA (DNA in tail as a % of total DNA) × TDx (tail length), TMOM levels have adjusted with TMOM of control.

[a] Age groups were categorized based on the average of workers' age.

2.7. Statistical methods

The JMP 5.0 (SAS Institute, Cary, NC) and Statistica 6.0 (StatSoft, Inc., Tulsa, OK) software packages were used for data management and statistical analysis. The Wilcoxon rank sum test was used to evaluate the differences in TMOM levels between different age groups, between participants with different working histories, between TMOM levels of workers in each type of plant, between the two groups of workers exposed to bottom vs. fly ash, and between different-length working periods.

3. Results

3.1. TMOMs of workers

There were no significant differences of the average TMOM between groups for age, smoking habit, alcohol-drinking habit, betel-nut chewing habit, or regular physical exercise of workers in both types of plants (Table 2). We found higher TMOM in fly ash treatment plant workers (7.55) than bottom ash plant workers (2.64). 22 workers were white collar and 56 were blue collar; significantly higher TMOM were found for the latter than the former (5.72 vs. 3.95). 64 workers (82%) responded that they usually exposed to bottom ash or fly ash, and a higher TMOM was detected in such people (5.71) than those who responded that they never exposed to bottom ash or fly ash (3.00) (Table 3). Four groups were categorized based on whether they ever exposed to ash or not and whether they used protective equipment against ash exposure. The highest TMOM was found in workers exposed to ash but did not use protective equipment (21.41); the second highest TMOM was in those exposed to ash but did use protective equipment (5.49). The lowest TMOM was in workers never exposed to ash regardless of whether they used protective equipment (Table 4). There were also four groups categorized based on whether they had ever exposed to ash and how long they had working in the plants. The highest level of DNA damage was found in workers exposed to ash and with a work history longer than 3.33 years (6.40); the second highest level was in those exposed to ash and with a work history less than 3.33 years (5.25). The lowest and, again, almost equal TMOM (3.24–3.26) were in those never exposed to ash regardless of the length of their work history (Table 5). Therefore, exposure to ash and not using protective equipment seemed to be the primary influence factors of DNA damage for ash treatment workers.

Table 3
The differences of DNA strand breakage levels (TMOM) between bottom and fly ash treatment plants, white collar and blue collar (Wilcoxon Rank Sum Test).

Variables	*N*	Mean ± standard deviation	Min–max	*p* value
Plants				
Bottom ash	37	2.64 ± 0.47	1.80–4.04	<0.0001**
Fly ash	41	7.55 ± 6.96	1.59–37.53	
Job styles				
White collar	22	3.95 ± 4.16	1.59–21.41	0.020*
Blue collar	56	5.72 ± 6.03	1.78–37.53	
Exposed to ash				
No	14	3.00 ± 1.29	1.59–6.59	0.024*
Yes	64	5.71 ± 6.05	1.78–37.53	

* $p < 0.05$;
** $p < 0.0001$.

Table 4
DNA strand breakage levels (TMOM) of workers expose to ash or use of protective equipment.

Expose to ash	Use of protective equipments	*N*	Mean	Min–max
Yes	No	1	21.41	–
Yes	Yes	60	5.49	1.78–37.53
No	No	8	3.17	2.27–6.59
No	Yes	8	3.31	1.59–6.87

3.2. Occupational exposure

When comparing particle concentration between samples inside the bottom ash and fly ash treatment plants, the higher particle concentration was found for coarse particle (10–21 μm) and fine particles (1–10 μm) in bottom ash plant than those in fly ash treatment plants, whereas for very fine particles (<1 μm) in between the two plants (Fig. 1). High percentage of very fine particles and coarse particles were found in fly ash treatment plants in bottom ash plant, respectively. The concentrations of chromium (Cr) and aluminum (Al) in diverse particles were showed in coarse, fine and very fine particles between the bottom ash and fly ash treatment plants (Fig. 2 and Fig. 3). The Cr and Al levels of fine and very fine particle in fly ash treatment plants were higher than those in bottom ash plant, whereas Cr and Al of coarse particles were higher in bottom ash plant than those in fly ash treatment plants.

3.3. Exposure index and TMOM

Further, we integrated the participants' occupational histories (whether they were exposed to ash, used protective equipment, and had ever worked in other similar factories; whether they had white collar or blue collar positions; and their length of employment and number of working hours per day) into an exposure index score that ranged from 0 to 9. TMOM levels were significantly higher in workers with exposure index scores >5 (67.5% of workers in fly ash plants and 29.7% in bottom ash plants) (Table 6) than in those with scores ≤5 (Fig. 4).

Table 5
DNA strand breakage levels (TMOM) of workers expose to ash and their working year (median = 3.33).

Exposure to ash	Working years	*N*	Mean	Min–max	*p* value
Yes	≥3.33	26	6.40 ± 6.47	1.78–25.03	0.292
Yes	<3.33	36	5.25 ± 5.94	1.92–37.53	
No	≥3.33	6	3.24 ± 1.86	1.59–6.87	
No	<3.33	10	3.26 ± 1.42	2.27–6.59	

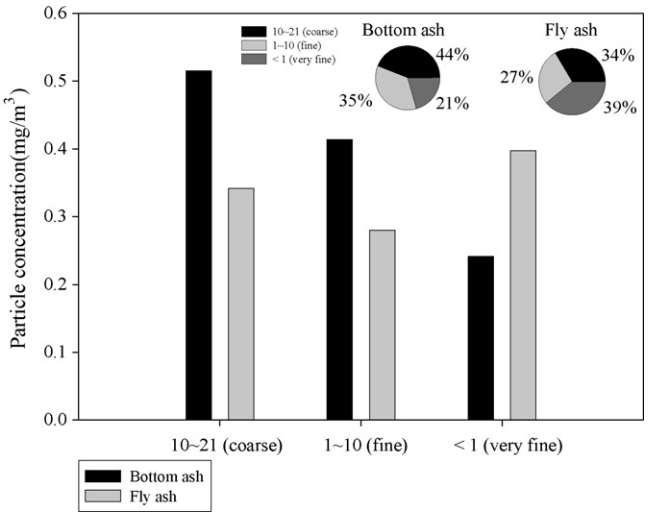

Fig. 1. Dust concentrations (μg/m^3) in particulates with different size interval between bottom and fly ash treatment plants.

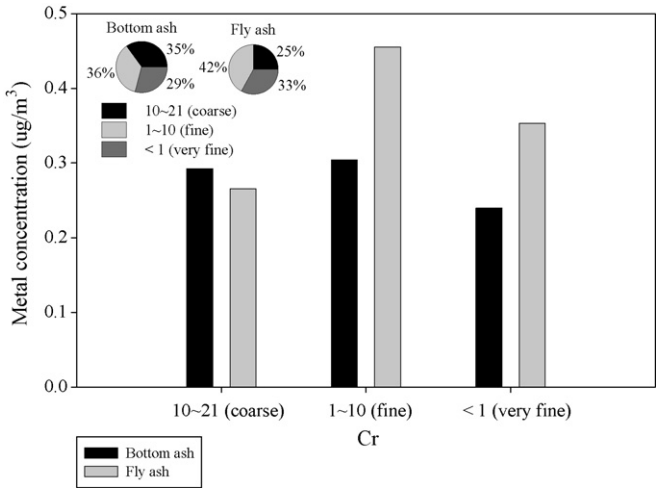

Fig. 2. Cr concentrations (μg/m^3) in particulates with different size interval between bottom and fly ash treatment plants.

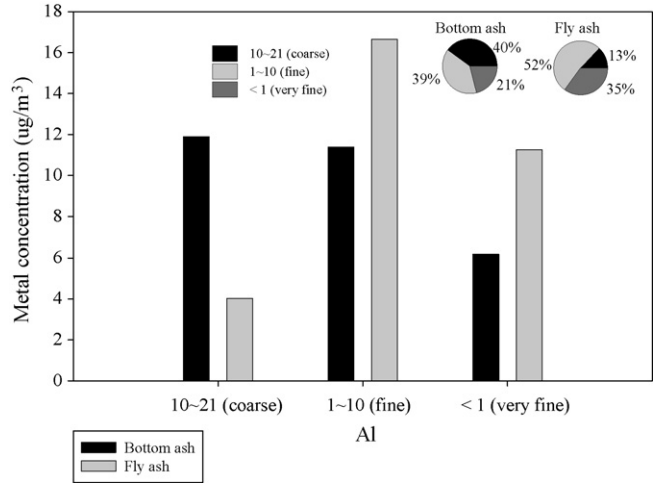

Fig. 3. Al concentrations (μg/m^3) in particulates with different size interval between bottom and fly ash treatment plants.

Table 6
Exposure index of workers between bottom ash and fly ash treatment plants.

	Index ≤5	Index >5	*p* value
Bottom ash	26 (70.27%)	11 (29.73%)	0.001*
Fly ash	13 (32.5%)	27 (67.50%)	

* $p < 0.05$.

4. Discussion

From the comet assays of 78 blue collar and white collar workers in fly ash and bottom ash treatment plants in Taiwan, we found that the tail moments in fly ash treatment plant workers were higher than those in bottom ash treatment plant workers. Lisk [16] reported that there might be much more arsenic, cadmium, nickel, lead, titanium, and zinc in fly ash and suspended particulates than in bottom ash. Yoo et al. [17] also identified more volatile metals such as cadmium, lead, and zinc, which they found were abundant in particulates emitted through stacks than in bottom ash. In addition, Chen and Lin [18] found that scrubber ash was more toxic than bottom ash, and that bottom ash was more toxic than cyclone ash. However, Kuo et al. [19] reported that iron, aluminum, copper, zinc, chromium, and lead concentrated in bottom ash, whereas cadmium existed primarily in fly ash. Xiao et al. [20] also reported that a significant amount of small pieces of metals, such as iron, copper, aluminum, lead, tin, zinc, and silver, were abundant in the bottom ash from municipal solid waste incineration. In addition, large quantities of PCDD/Fs were leached from fly ash but not from bottom ash [21]. Most studies have reported that metals and PCDD/Fs were more abundant in fly ash than in bottom ash [16,17]. In this study, except for the metal components between the bottom ash and fly ash treatment plants, the air samplings inside the two plants also suggested that the particle size might be important to affect the workers inhaling the metal into the human body and finally caused to the DNA damage [22,23]. Ontiveros et al. [22] have suggested that Cu, Zn, Cd, and Pb were predominant in small size particulate, by contraries, Al, Ni, Pb, Fe, and Cr levels were higher in the small size particulates than those in the large one. Chimenos et al. [23] also showed the high variation of mass concentration with different particle size in bottom ash and fly ash industries. Meanwhile, there will increase 13% relative mortality rate while people expose to 2.5 μm particulate, as well as 0.3% increasing while people exposed to 2.5–15 μm particulate [24]. Therefore, fly ash treatment plant workers seem to be exposed to more toxic hazards than bottom ash

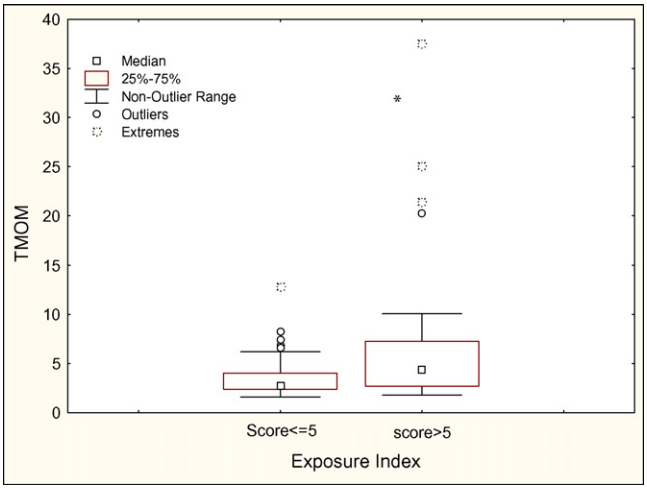

Fig. 4. DNA strand breakage levels (TMOM) of workers based on their exposure index: Score ≤5 or >5 (**p* value < 0.05, significant difference between the two groups).

treatment plant workers, and the differences in oxidative damage between bottom ash and fly ash workers treatment plant workers appear consistent with this notion and previous reports.

Furthermore, the present study showed that exposure to ash and not using protective equipment seemed to be the main influence factors affecting the level of DNA damage in ash treatment plant workers. Most of the participants in this study wore cotton or active carbon masks. The aerodynamic diameter is 0.1–2 μm of particle has good penetrability to cotton or active carbon masks. Personal protective equipment is not so effective for protecting workers against occupational hazards [25]; meanwhile, risk assessments of exposure to occupational hazards might be influenced by exposure scenarios for each worker, including different workplaces, individual physical fitness, and how well the personal protective equipment is maintained and stored [26]. Thus, both bottom and fly ash treatment plant workers should be especially careful to select the basal respiratory protection suited for the hazards they are exposed to.

The second most important factor affecting the level of DNA damage in ash treatment plant workers was how long they had been working in such a plant. Further, the integrated exposure score also indicated significantly higher DNA damage for workers with scores >5 than those with scores ≤5. Therefore, the data indicated that an elevated DNA strand breakage may be expected in fly ash treatment plant workers with more hazardous exposure.

5. Conclusion

The present study shows that workers at fly ash treatment plants had significantly higher DNA damage than those at bottom ash recovery plants, as well as the air samplings for particle mass, Cr, and Al concentrations. The fine particle in the two plants might be suggested to responsible for the workers inhaling the metal into the human body and finally caused to the DNA damage. It also showed that workers exposure to ash and not using protective equipment seemed to be the main influence factors affecting the DNA damage in ash treatment plant workers.

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