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ASSESSMENT OF PARTICULATE MATTER (PM_{10}) AND POLYCYCLIC AROMATIC HYDROCARBONS LEVELS AT VARIOUS SITES IN FAISALABAD AND THEIR POTENTIAL TOXICITY

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Based upon the presence in environmental samples and toxicity, USEPA has a set of chemicals generally termed as priority pollutants. The objectives of present work were to find out particulate matter (PM_{10}) concentrations in Faisalabad city, identify most commonly reported 16 priority polycyclic aromatic hydrocarbons (PAHs) and quantifying the DNA damage caused by PAHs present in PM_{10}. Four sites from the Faisalabad city; Chenab Chowk (CC), Government Transport Service Chowk (GTSC), General Bus Stand (GBS) and Allied Chowk (AC) were selected due to high traffic loads at these points. Average PM_{10} concentrations at these sites were 372, 283, 223 and150, µg and m^{-3}, respectively when measured with high volume sampler and maximum concentrations were 501, 456, 625 and 271 with Casella Microdust Pro™ sampler. Ten out of 16 priority PAHs were identified using GC/MS technique. These were naphthalene, acenaphthylene, acenaphthene, anthracene, benzo(e)pyrene, chrysene, flourene, fluoranthene, naphthalene, phenanthrene and pyrene with varying concentrations. The DNA damage was detected through Comet assay. The significant DNA damage was seen in exposed cells (22%) as compared to the control. The PM_{10} concentrations were higher than the EPA designated limits of 150 µg m^{-3}. So there is urgent need to reduce emissions to meet the set criteria for the air quality in order to minimize damage to the environment as well as human health.

Keywords: Particulate matter**,** PAHs, Comet assay, sonication, genotoxicity

INTRODUCTION

Particulate pollution is basically caused by the suspended particles of respire-able size in the air. The most common anthropogenic source contributing particulate matter (PM) to the urban atmosphere is the vehicular emissions. It has gained the importance due to occupational exposure for drivers and public exposure for the people using these vehicles for transportation and road sides for daily activities (Devi *et al.,* 2009; Zuurbier *et al.,* 2010; Chio *et al.*, 2012). During the days of pilgrimage, the PM concentrations ranged 158.5–444.5 µg m^{-3} at Muzdalifa and Arafat, Jeddah, Saudi Arabia due to the highest flow of vehicles. These values were higher than the maximum limit of 150 µg m^{-3} designated by EPA while the maximum level of PAHs reported was 5 ng m^{-3} (Elassouli, 2011). Occupational exposure might have temporal variations. The exposure concentrations of PM were the highest in the morning and lowest in the noon and, similarly higher in week days as compared to the weekends (Bashyal *et al.,* 2008). The concentrations of priority pollutants depend upon the number of vehicles and presence of other activities like incinerators or industry (Elassouli *et al.,* 2007). Generally, PM concentration is higher in underground pathways and tunnels due to the poor circulation of air. PM gets suspended and can be more harmful for the human health, especially due to its tendency to get accumulate in the lungs (Jung *et al.,* 2012).

Toxicity of PM also depends upon its size. $PM_{2.5}$ is more damaging to human health as compared to PM_{10} (Buschini *et al.,* 2001). PM_{10} concentration tends to be considerably high at busy streets as compared to the city backgrounds and suburban locations (Boogaard *et al.,* 2010). PM_{10} concentrations are higher in the underground stations as compared to the $PM_{2.5}$. The coarse to fine particle ratio is higher at busy roads with congested traffic as compared to the roads having continuous traffic (Strak *et al.,* 2011). PM_{10} concentration in metro system tends to be higher than that at the streets and the levels are decreased when the tracks and walls of metro stations are washed (Nieuwenhuijsen *et al.,* 2007). Vehicular shut downs have considerable impact on the levels of PM as shown in a study done in Katmandu, Nepal. The highest concentrations recorded were 290 and 236 µg m^{-3} at two sites with the highest traffic loads and the lowest were 48 µg m^{-3} reported in rural areas. The concentrations during the strikes were considerably low (Fransen *et al.,* 2013). PM concentrations show a sudden decrease when the natural phenomenon like dust storms are not occurring, the precipitation rate is increasing and the strategies to lower down the emissions are being implemented (Cheng *et al.,* 2013). Alam *et al.* (2011) conducted a study to estimate particulate matter in four big

cities of Pakistan i.e. Karachi, Rawalpindi, Lahore and Peshawar. The highest concentration (540 µg m^{-3}) was found in Peshawar and the lowest in Lahore (198 µg m^{-3}).

PM might have significant amounts of PAHs adsorbed to the particles. The PAHs are capable of producing DNA damage in the human cells. The PAHs cause more damage in winter due to less photolysis as compared to the summer (Buschini *et al.,* 2001). The PAHs extract is capable of producing DNA damage in lung epithelial cells (Oh *et al.,* 2011). The PAHs extract from a more polluted area causes a greater damage to the cells as compared to the extract from a less polluted site. PAHs extract could not be directly mutagenic and might need metabolic activation (Elassouli, 2011). In this context, the objectives of present work were; 1) To find out particulate matter (PM_{10}) levels at different locations in Faisalabad, Pakistan, 2) Qualitative and quantitative analysis of priority PAHs present in PM_{10} and, 3) to find the DNA damage potential of PAHs from PM_{10}.

MATERIALS AND METHODS

***Sampling location and equipment*:** Faisalabad city is present in the North East of the Punjab Province (31°25′4.8″N, 73°4′44.4″E), Pakistan. Four sites based on high traffic intensity were selected for the sampling. These include Allied Chowk (AC), Chenab Chowk (CC), General Bus Stand (GBS) and the Government Transport Service Chowk (GTSC). The sampling was done with the help of two types of samplers, Casella Microdust Pro™ sampler (manufactured by Casella, Canada) and the high volume air sampler (Wedding and Association Incorporation, Critical High Volume Sampler, US patent#4,649,760). The Microdust Pro™ sampler having the size selective adapter using poly-urethane foam filters for PM_{10}. This is a portable sampler and gives the instant concentration of target particulate matter. The high volume sampler is recommended by Pak-EPA or US-EPA for the measurement of the particulate matter. Glass fiber filters were used to find out the concentrations using gravimetric method.

***Sampling and measurements of PM_{10}*:** Both the samplers were installed simultaneously on each site to find out the concentration on the consecutive days for one day sampling of each site. The sampling period was 8h a day for each site. The Casella Microdust Pro™ sampler was operated through rechargeable battery while the high volume sampler was operated with the continuous DC supply through the generator. The generator was set in a manner so that it may not be able to produce any smoke itself to avoid the chance of error.

The Casella Microdust Pro™ sampler shows the immediate concentration within the air on its screen depending upon the probe and filters used. The high volume sampler requires gravimetric measurements. The filters were pre-weighed before the installation and after the sampling with the help of top loading balance. The weight difference then divided by the total volume of air sampled gives the total concentration of PM_{10}. The formula used is;

$$\text{Total concentration} = \frac{W2-W1\times 1000000}{Total\ volume}$$

Whereas total volume is measured by the following formula:

Total volume = Volume of air/minute Time of sampling

***Extraction of PAHs and analysis*:** The poly urethane filters in case of Casella Microdust Pro™ were desiccated before and after the sampling for a minimum of 18h and soon after the sampling were placed in the freezer at -20°C. The glass fiber filters from the high volume sampler were heated in oven at 103°C for 30 min and then after sampling were preserved in the freezer at -20°C. For analysis of 16 priority PAHs, the poly-urethane foam filters used in the sampling from CC in the morning, noon and evening were selected. These filters were then sonicated using dichloromethane as an extracting solvent in order to extract the PAHs. This step was performed in a beaker using 50 mL of dichloromethane for 30 min each. After 30 min, the filters were taken out of the beaker and the liquid was allowed to dry in air. Near the evaporation, 0.1 mL of acetonitrile was added to prevent the loss of hydrocarbons.

After evaporation, the colloidal solution gained was immediately dissolved in 1 mL of dimethyl sulfoxide and then filled in Eppendorf tubes and were stored at 4°C. Due to the unavailability of the standards within the country, the samples were sent to ALS, Canada for analysis. The analysis was done using Agilent 5975 GC/LRMS (Gas Chromatography coupled to Low Resolution Mass Spectrometry). The analytical method was as per California Air Resources Board method 429, isotope dilution technique showing recovery of ≥91% for the targeted PAHs.

***Comet assay*:** The DNA damage potential was checked using comet assay (Singh *et al.,* 1988). The human peripheral blood was taken from the healthy donors and was exposed to the extracted PAHs at concentrations of 150, 250 and 350 µg mL^{-1} along with the addition of S9 liver fraction in an incubator for 18h. The slides were prepared in single layer with 1% of the low melting agarose. For this purpose, 0.6 mL of low melting agarose per slide was placed and 0.2 mL of blood cells was added on to it. The slides were allowed to settle down and solidify. After that the slides were placed in lysing solution (NaCl 14.6g, EDTA 3.72 g, Trizma 0.12g, NaOH 0.88 g, Triton X 1% and DMSO 10%) overnight. The slides were then suspended in the electrophoresis solution (NaOH 12g, EDTA 0.75g and pH> 13) for 20 min and were electrophoresed for 20 min at 15V and 155mA. After washing with the neutralization buffer, the slides were stained with ethidium bromide and were seen under the microscope (Leica DM RXA, Meyer Instruments, Inc. USA) to analyze the DNA damage.

***Statistical analysis*:** The statistical significance of results was checked by using Student's T-test and standard deviation. Statistically significant differences were reported when the probability of the result assuming the null hypothesis was $p< 0.05$.

RESULTS

***PM_{10} concentrations determined with Microdust Pro™ sampler*:** The concentration of the PM_{10} taken from the Microdust Pro™ sampler in the morning, noon and evening are presented in Figs. 1, 2 and 3, respectively. At one specific interval e.g. morning, there was little variation among different values.

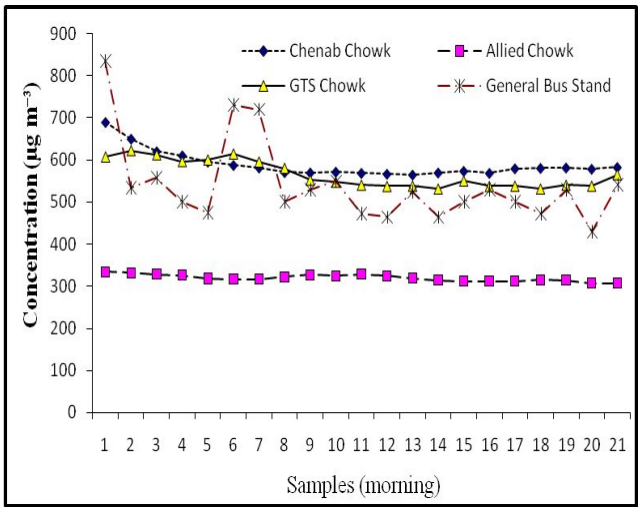

Figure 1. PM_{10} concentrations in the morning with Casella Microdust Pro™sampler.

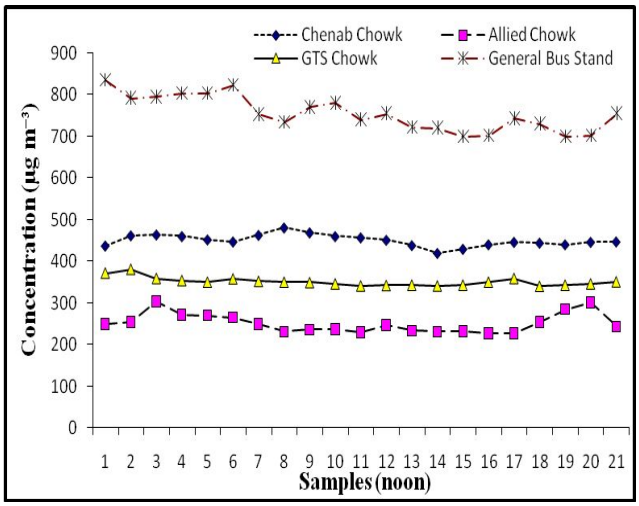

Figure 2. PM_{10} concentrations at noon with Microdust Pro™ sampler.

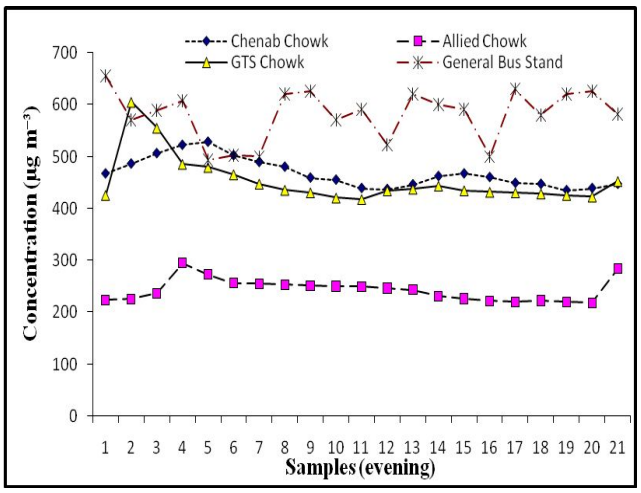

Figure 3. PM_{10}concentrations in the evening with Microdust Pro™ sampler.

The average concentrations were higher in the morning as compared to the concentrations in the noon and evening. These were 588, 319, 565 and 541 µg m^{-3} at CC, AC, GTSC and GBS, respectively (Table 1). In the noon, the concentrations were lower than the morning and the evening at CC and GTSC. These were 450 and 350 µg m^{-3} in the noon and, 467 and 452 µg m^{-3} in the evening respectively (Table 1). At GBS, the concentrations were higher in noon as compared to the morning and evening. At noon the value was 755 µg m^{-3} while in evening, it was 580 µg m^{-3} (Table 1). At GBS, the concentrations in the evening are a little higher than the morning. At AC, the concentrations were almost same at noon and the evening, i.e. 251 and 242 µg m^{-3}, respectively (Table 1). The highest per day average concentrations were seen at the GBS that were 625 µg m^{-3}. The concentration at CC was 501 µg m^{-3}. At GTSC, the value was 456 µg m^{-3} and the lowest was at AC, i.e. 271 µg m^{-3}. The differences in the concentrations are related to the traffic loads, the capacity of the roads, roads surrounded by the shops, burning of kilns, weekend or week days, precipitation and the direction of the air. These all conditions are the factors that usually control the level of particulate matter.

***PM_{10} levels determined with high-volume air sampler*:** The concentrations measured by the high volume sampler shows highest concentration at CC that was 372 µg m^{-3}, followed by the GBS that was 283 µg m^{-3}, the GTSC had 233 µg m^{-3} and the lowest levels at AC 150 µg m^{-3} (Table 2). When compared with the results of Microdust Pro™, considerable differences were recorded. Overall values were low with high volume air sampler as compared to Micrdust Pro™. The difference was 130 and 121 µg m^{-3} at CC and AC, respectively. However, the differences at GTSC and GBS were relatively high i.e. 233 and 342 µg m^{-3}.

Table 1. PM10 concentrations determined with Casella Microdust Pro™ sampler.

Location	Morning	Noon	Evening	Mean
CC	588.14±6.83 b	450.10±3.12 d	467.71±6.14d	501.98±8.42B
AC	319.48±1.72 f	251.48±5.09 g	242.67±4.81g	271.21±4.96D
GTSC	565.24±7.07 bc	350.95±2.28 e	452.33±10.22d	456.17±11.86C
GBS	541.52±21.76 c	755.19±9.14 a	580.52±10.70b	625.7±14.54A
Mean	503.60±13.19 A	451.93±20.89 B	435.81±14.01C	

Values are presented as means ± standard deviations. Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 2. PM_{10} concentrations taken from high volume air sampler.

Location	Weight of filter		Difference (g)	Total volume (m^3)	PM_{10} (µg m^{-3})
	Before (g)	After (g)			
CC	2.81	3.10	0.25	672	372.0
AC	2.79	2.89	0.10	672	150.0
GTSC	2.81	2.96	0.15	672	223.2
GBS	2.83	3.02	0.19	672	283.0

The values are the sum of 8h sampling period.

***PAHs concentrations in PM_{10}*:** The analysis of the extracts for PAHs showed the detection of 10 polycyclic aromatic hydrocarbons out of 16 priority pollutants (Fig. 4) designated by EPA namely; acenaphthylene, acenaphthene, fluorine, phenanthrene, anthracene, fluoranthene, naphthalene, pyrene, chrysene, denzo (e) pyrene. Benzo(A)anthracene also appeared on the graph but its values were below detection limits.

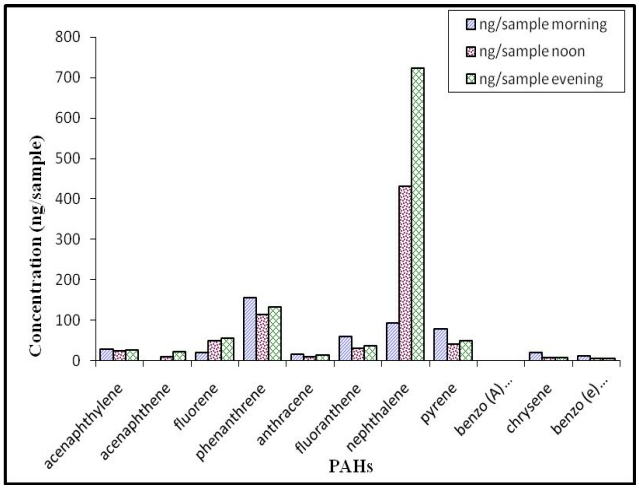

Figure 4. Concentrations of polycyclic aromatic hydrocarbons in the PM_{10} extract.

The total amount per day detected was 2272 ng and the total amount of the polycyclic aromatic hydrocarbons was 37 ng m^{-3} (Fig. 4). The most dangerous pollutant benzo (a) pyrene was not detected in the samples. The permissible limits for the PAHs are 0.001 mg m^{-3}. The detected values are within the permissible limits as set by the EPA. The highest concentration was of naphthalene 62.34 ng that becomes 55% and the lowest were of chrysene with 1.0695 ng that becomes 0.95% of the total concentration 111.7 ng (Fig. 4).

***DNA damage assessments*:** Control cells with no exposure had no DNA damage (Fig. 5a) whereas considerable damages was recorded with cumulative exposure of blood cells to PM_{10} extract for PAHs (Fig. 5b) and the only naphthalene (Fig. 5c).

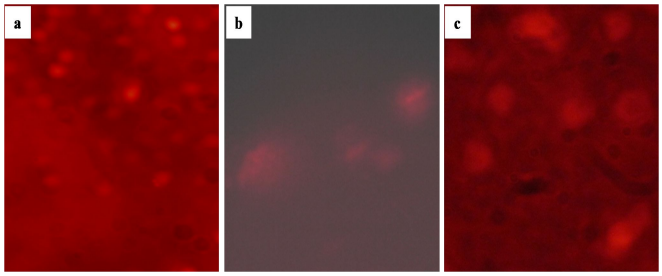

Figure 5. DNA damage upon exposure to PAHs. a) Control cells without exposure and no DNA damage; b) cells with DNA damage caused by the PAHs; c) Cell damage caused by the naphthalene.

In case of extracted polycyclic aromatic hydrocarbons, the damage was seen at a concentration of 350 µg mL^{-1}. The extract caused damage in 7 out of 30 cells in the slide with the exposure of 350 µg mL^{-1}. The extract caused DNA damage to 22% of the blood cells. The naphthalene started to

cause damage in the cell at the concentration of 250 µg mL^{-1}. The doses lower than this did not cause any damage.

DISCUSSION

***PM_{10} concentrations*:** The results show that the concentrations taken at different location varied from each other. The reason for the differences can be the spatial variations and the type of activities other than vehicular circulations and the day of the week. Generally, these factors determine the concentration of particulate matter (Alam *et al.,* 2011; Bashyal *et al.,* 2008; Elassouli *et al.,* 2007). The highest mean concentration of PM_{10} was found at the GBS. This could be due to heavy traffic as it is a bus stand, emissions from the generators during the load shedding, and auto rickshaws. The concentrations in the noon are considerably high due to the higher number of working generators at the time of sampling and in the evening it was again close to the levels in the morning. The second highest concentrations taken from the Microdust Pro™ sampler were at the CC. This point is close to the university gate where pick and drop activity for the students, heavy traffic load and emissions from the generators from nearby shops could potentially contribute to high levels of PM_{10}. At GTSC, the traffic is heavy but the sampling site did not had any kind of shops and, the roads are comparatively wide and an underpass has separately been made for the buses to leave for different locations and the area is not congested. The concentrations at AC are the lowest because the sampling day was Sunday and the traffic was low at the site as compared to the week-days. The results taken from the Microdust Pro™ can be related to the study of Bashyal *et al.* (2008). They reported higher concentrations of PM_{10} citing the heavy traffic and re-suspension of the PM_{10} in the air that were settled down on the roads due to the circulation of traffic and sweeping on the roads in the morning as possible reasons. In noon the traffic flow is less as compared to the morning and evening and the concentrations are lower in weekends as compared to the week days. When measured with the high volume sampler, the 8h average showed the highest concentrations at CC. The concentrations at the GBS are lower than CC because there was a slight rain fall resulting into a little decrease in previous suspended particles before the placement of sampler and the sampler gathered only those particles that were generated after that period. The concentration at CC, GTSC, GBS and AC were 372, 283, 223 and 150 µg m^{-3}, respectively. The average concentration from four sites was 256.75 µg m^{-3}. The results can be compared with the study by Alam *et al*. (2011) in which the concentration of PM_{10} in Karachi, Lahore, Rawalpindi and Peshawar were reported as 270,198,448 and 550 µg m^{-3}, respectively. So the concentrations in Faisalabad are higher than Karachi and Lahore and less than Rawalpindi and Peshawar. The result showed that the PM_{10} concentrations mainly depend upon the traffic flow as reported in different studies (Elassouli, 2011; Fransen *et al.,* 2013; Zuurbier *et al.,* 2010). Increase in PM_{10} concentrations is also associated with various factors like traffic rush hours, congestions, diesel operated generator during power black outs, construction activities and meteorological parameters like temperature, relative humidity and wind speed. A recent study, Al-Jallad *et al.* (2013) and references there in, investigated the influence of meteorological variables on PM_{10} concentrations. A positive correlation is found among PM_{10} concentration, temperature and humidity less than 25%. While the PM_{10} concentrations decrease significantly with the increase of humidity greater than 25%. In our study, effects of temperature and humidity could be negligible as all the measurements were made in the same season and consecutive days.

The difference in the concentrations taken by both samplers is obvious because the Microdust Pro™ gives the immediate concentration of the desired size of the particulate matter on sampling site. The values were higher as compared to the high volume sampler that first intake flow separate the desired size fraction through cyclone and then collect them on the filter paper so there might be chances of loss. The concentrations of PM are higher than designated by the EPA.

***Contents of priority pollutants*:** The PM_{10} extracts showed the presence of 10 EPA designated priority pollutants that are potential carcinogens. The concentrations are higher than the studies that were consulted as reference (Elassouli *et al.,* 2007; Elassouli, 2011). The method used for the extraction was different. The method followed in these studies used Soxhlet apparatus for the extraction. The method used in our study was sonication using the dichloromethane as the solvent. It showed better result than the Soxhlet method and also the method 429 designed by Canadian standards for extraction that was used by the analytical lab to extract the PAHs from the filter paper used for sampling. The sampling sites had similar characteristics for the PM sources so any of the sites can be selected to have extracts for the analysis. If the sites have different sources of PM emissions, then the extract should be taken from each sampled site to show the differences as described in literature (Elassouli *et al.*, 2007).

***DNA damage*:** The results showed that the PAHs produced the dose dependent response as the damage started at the maximum concentration. The damage can be much more significant than that reported in the study as the samples are taken in summer and are less damaging than the extracts taken in winters as reported by Buschini *et al.* (2001). Moreover, no comet was seen without S9 fraction and the damage was seen when the metabolic activation was introduced, so PAHs are not direct mutagens and require activation as reported in literature (Elassouli, 2011).

***Conclusions and perspectives*:** The concentrations of the PM_{10}were higher than the set criteria of the Pak-EPA. The

presence of priority pollutants and confirmation of their potential to cause genotoxic effects create alarming situation. So serious considerations should be given to reduce the emissions and improve the air quality to handle the risks to environment and human health. There is also need to consider $PM_{2.5}$ concentrations at these locations as this fraction is reportedly more toxic as compared to PM_{10} (Javed, 2014).

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