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Research Article

**HEMATOLOGICAL STUDIES AMONG GURHAKU SMOKERS
IN SELECTED AREAS OF BALOCHISTAN****Waheed Ahmed^{1,2}, Ashif Sajjad², Mohammad Azam², Zahid Mehmood² and Khalid Mahmood^{2*}**¹ Institute of Biochemistry, University of Balochistan, Quetta^{1,2} Department of Chemistry, Government College of Technology (GCT), Quetta**Abstract:**

Gurhaku, is one of the newly emerging smoking materials, prepared by mixing tobacco dust and molasses smoked in hookah. Tobacco smoking causes human health problems and has got a close association with the environmental contamination. In the present study Hematological parameters of Gurhaku smokers were analyzed by the hematological analyzer while the elemental analysis of waste water were carried out by using atomic Absorption Spectroscopy. The results of the current study revealed that the Ghurhaku smokers have reduced levels of Hb and have significantly higher platelet and WBCs count including lymphocytes and granulocytes. However, the RBC count, Mean Corpuscular volume (MCV), and Hematocrit percentage (HCT %) did not show any significant change. The elemental analysis of the Gurhaku smoked Hookah smoked water revealed the concentration of Pb was higher than WHO permitted value. The concentrations of Mn, Cd, Zn, Fe, Cu, Ca and Mg have lower values than the WHO permitted values. It is suggested on the basis of this study that the complete blood count (CBC) of the Gurhaku smokers be dealt with caution in hospitals.

Key words: Ghurhaku, Heavy metals, hematology, Atomic absorption Spectroscopy**Corresponding Author:****Khalid Mehmood,**

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INTRODUCTION:

Tobacco is a product prepared from the leaves of the tobacco plant. It belongs to the genus of the Solanaceae (*nightshade*) family having more than 70 species. The main commercial crop is *N. tabacum* and *N. rustica*, cultivated throughout the world. Dried tobacco leaves are mainly used for smoking in cigarettes, cigars, bidi, pipe tobacco, and flavored tobacco. They can also be consumed as snuff, chewing tobacco, dipping tobacco and as moist powder tobacco [1].

Gurhaku, which is used for smoking in hookah, is one of the newly emerging smoking materials, prepared by mixing tobacco dust and molasses. It is gaining popularity in province Sindh and Baluchistan because of its better taste and cheap price as compared to other tobacco brands. According to a report (by Aziz Sanghur, News One Karachi) about 0.3M women are addicted to Gurhaku in Pakistan who mainly belong to province Sindh and Baluchistan. On average an addict smokes some 4 pieces of Gurhaku/day and costs about 17 million rupees/annum. Approximately some 25 kg of tobacco dust is mixed with some 100 liters of molasses in a large container maintaining a rough ratio of 1:4. Once mixed well, rough rectangular shaped Gurhaku pieces are made and left for dry followed by their storage in wooden containers for the supply to the general stores.

Smoking devices need the attention of environmental monitoring studies. The use of water pipe for the purpose of smoking tobacco is an ancient tradition in many parts of the world. The water pipe bears a number of different names depending on region and origin i.e. Hookah, Goza, Hubble and Bubble, Narghile and Shisha. The composition of tobacco mixtures used in water pipes vary widely and availability of particular type of tobacco [2]. In sub-continent Hookah is one of the commonest smoking devices after cigarettes. It is smoked in several South Asian and Middle East countries, but has gained popularity among a number of western countries with a name of Shisha [3, 4].

There exist great differences between water pipe and cigarette smoking. As per the report of WHO, by using water pipe in a typical session of smoking which often lasts up to an hour, results in 100–200 times more volume of smoke inhaled in a single cigarette session which contains a mixture of toxicants i.e. carbon monoxide and metals.

The *Nicotiana tabacum* leaves contain a chemical called nicotine, which is the main cause of addiction of tobacco, is harmful to humans [5]. Tobacco contains

over 19 known cancer causing substances and about 30 metallic compounds comprising heavy metals. The Tobacco plants absorb, accumulate and transport heavy metals like cadmium (Cd), lead (Pb), Iron(Fe), chromium (Cr) and Copper (Cu) from the soil into leaves.

Tobacco smoking causes human health problems and has got a close association with the environmental contamination. World Health Organization named tobacco as one of the world's single greatest preventable causes of death and reported that about 12% of all adult deaths could be attributed to tobacco smoking. Five million deaths were a direct result of the use of tobacco smoking or smokeless forms which is translated to be one death in every six second. Tobacco smoking generates most of environmental and health issues [6,7].

No previous selective studies have been done on Gurhaku. Considering the above facts the present investigation was designed to monitor heavy metals in Gurhaku smoked wastewater by using Atomic Absorption Spectroscopy (AAS) and effects on hematological parameters.

METHODOLOGY:

Collection of Blood samples

A detailed questionnaire was provided to the participants to explain them about the purpose of the study and after getting their consent, blood samples were collected in Vacutainer tubes containing EDTA with the help of trained medical professionals under the supervision of tribal elders. The containers were labelled, and were immediately stored in the blood transport boxes making sure to provide a temperature controlled environment (About +4 Celsius) to the samples, wasting no time, the samples were rushed to laboratory for the complete blood count (CBC) analysis via automated hematological analyzer.

Collection of Hookah water samples

Hookah water samples (this includes Gurhaku smoked distilled water which was provided for the hookah to the smokers) were collected in the pre-cleaned containers and sealed from at least 4 sampling location from the Lasbela and Kalat district of Baluchistan. The areas include Hub, Munghachar, Mastung and Quetta etc. The samples were stored in the refrigerator for further use.

Ethical committee approval

In order to collect blood samples from the selected areas of Balochistan, approval was taken from the ethical committee of the institute of Biochemistry, University of Balochistan

Selection Criteria for the blood donors

1. Exclusive Gurhaku smokers for at least last three years.
2. Age should be between fifteen to fifty years.
3. The selection of blood donors was done randomly.

Exclusion Criteria

The candidates with self-reported acute illness, heart diseases, chronic liver, lung diseases, kidney failure, malignancy, diabetes mellitus, obesity, alcohol consumers were excluded during the study.

Digestion of Hookah water samples

To prevent the interferences in analysis and complete removal of impurities from the samples, the digestion of Gurhaku smoked water samples were carried out by using concentrated (Nitric acid) HNO_3 . 3 mL of conc. HNO_3 was mixed into 50 mL of sampling water into a pre-cleaned flask. The mixture was heated on a hot plate under the fuming cupboard until we were left with about 20 ml of this mixture. Cooled the mixture, added 3 mL of conc. HNO_3 and started heating the flask on the hot plate until a clear solution was obtained, diluted the solution by using distilled water into a 100 mL of volumetric flask. The final solution was stored in the refrigerator for further use [8].

RESULTS AND DISCUSSIONS:

Hematological parameters

The current study was designed on the Ghurhaku smokers of province Baluchistan whose ages were in between fifteen to fifty. During the study the blood samples of ninety nine Gurhaku smokers (study group) and twenty non-smokers (control groups) who were not using any type of smoking material, were analysed in Haematological analyser. Haematological parameters analysed during the study are given in the table-1. Gurhaku smokers were divided based on their ages starting from fifteen and went on as multiples of three i.e. 15-18, 18-21 and so forth till 45-48.

The result of the current study revealed that the Ghurhaku smokers show comparatively lower levels of Hb. The concentration of Hb ranged from 9.56 to 12.1 g/dl whereas the concentration of haemoglobin in the study group was 13.6. Only the age group 19-21 had significantly lower Hb levels (9.56 g/dl) however, the rest of the age groups were found to have slightly reduced Hb levels. This observation is in agreement with the observations of P Jaganmohan et al [9] and Verma R.J et al [10] who found significantly lower levels of Hb levels among gutka (smokeless tobacco) users and cigarette smokers respectively. Slight reduction in the Hb concentration among Gurhaku smokers is probably due to highly reduced quantity of tobacco (nicotine) in the Gurhaku.

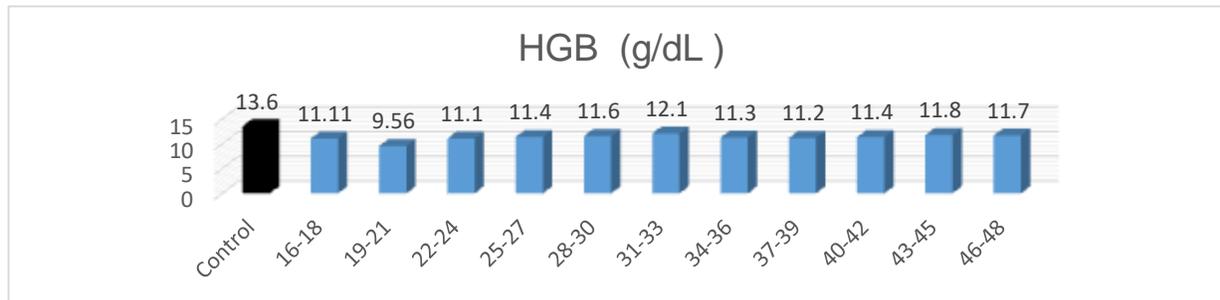


Fig.1: Various age groups (in years) and their HGB concentrations

The levels of WBCs were found to be higher ranging from 7.0 to 12.6 billion/liter whereas the levels of WBC in the control group was 8.2 billion/liter. Similar results were found by P Jaganmohan et al and Verma R.J et al who found significantly higher levels of WBC among gutka (smokeless tobacco) users and cigarette smokers respectively.

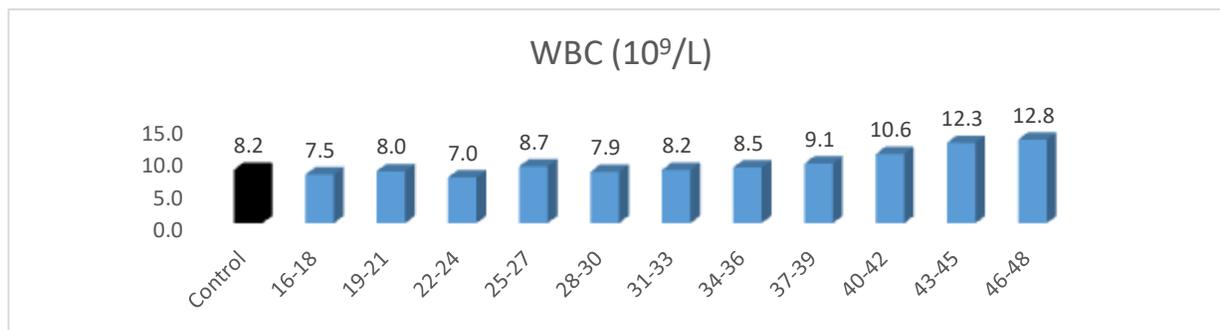


Fig.2: Various age groups (in years) and WBC concentrations.

Similarly the levels of lymphocytes and granulocytes were found to be higher. Increase in the WBCs count is possibly the result of inflammatory responses and it might be speculated that the increase in the WBC count is due to the tissue damage in the body.

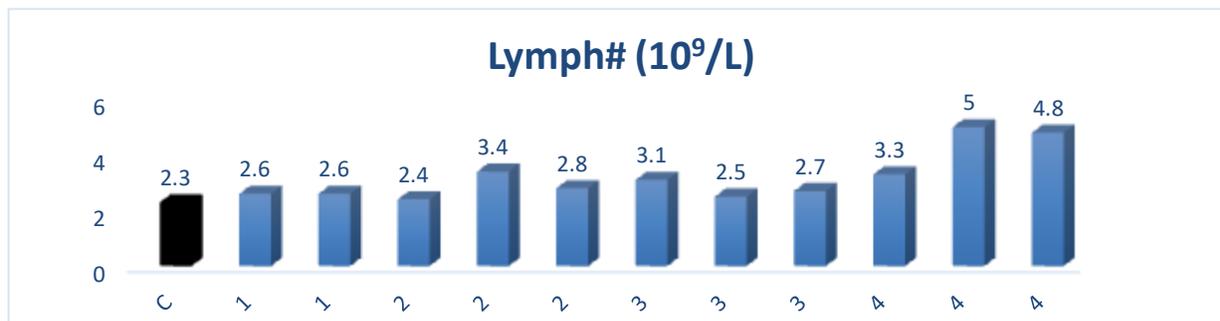


Fig.3: Various age groups (in years) and lymphocytes concentrations



Fig.4: Various age groups (in years) and Granulocytes concentrations

However, the results of RBC were almost normal ranging from 4.28 to 5.12 ten trillion/Liter and the RBC count in the control group was 4.9 (10¹²/L) which is in disagreement with the observations made by DR. B.K.Binawara et al [11] on smokeless tobacco who found that the RBC count was increased by consuming smokeless tobacco whereas Verma R.J et al found significantly lower levels of RBC among cigarette smokers. Normal RBC count among Gurhaku smokers indicates that neither haematopoiesis is disturbed nor abnormal destruction of RBC is going on.

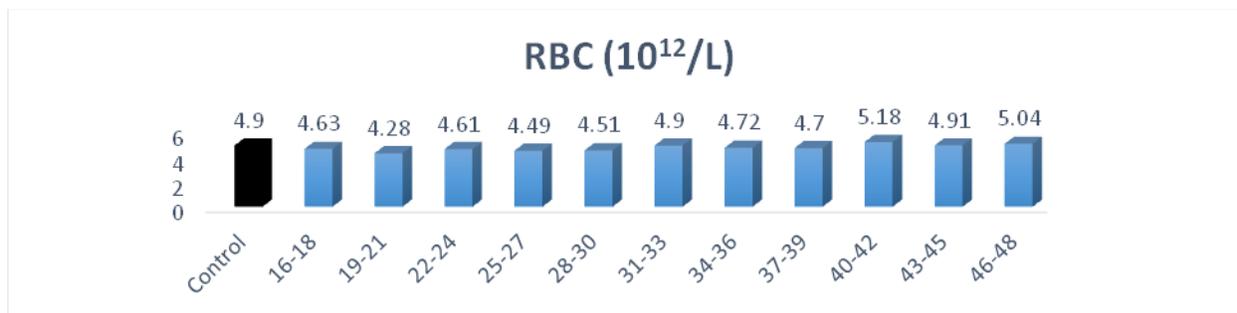


Fig.5: Various age groups (in years) and their RBC concentrations

Platelets count was found to be slightly higher in the Gurhaku smokers (ranging from 306-397 (10⁹/L)) than the control group (288 (10⁹/L)). Similar results were found by Verma R.J et al who reported significantly higher platelet count among cigarette smokers.

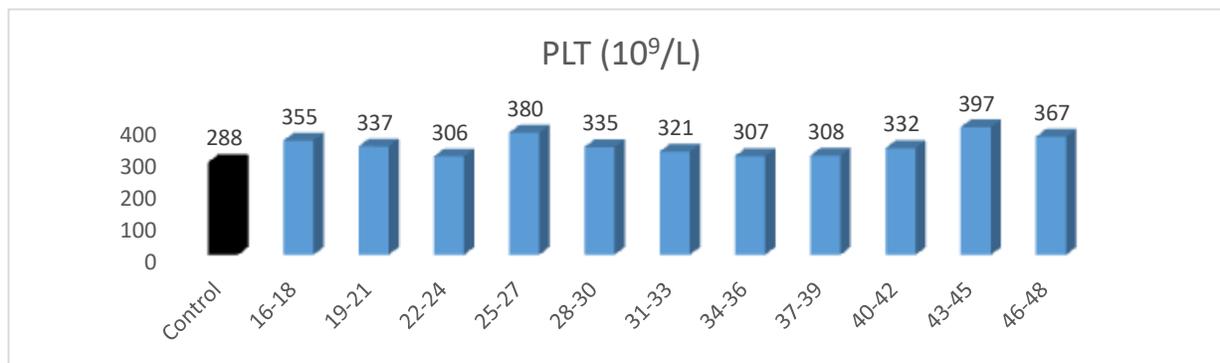


Fig.6: Various age groups (in years) and Platelets concentrations

Mean Corpuscular volume (MCV) and Haematocrit percentage (HCT %) did not show any significant change.

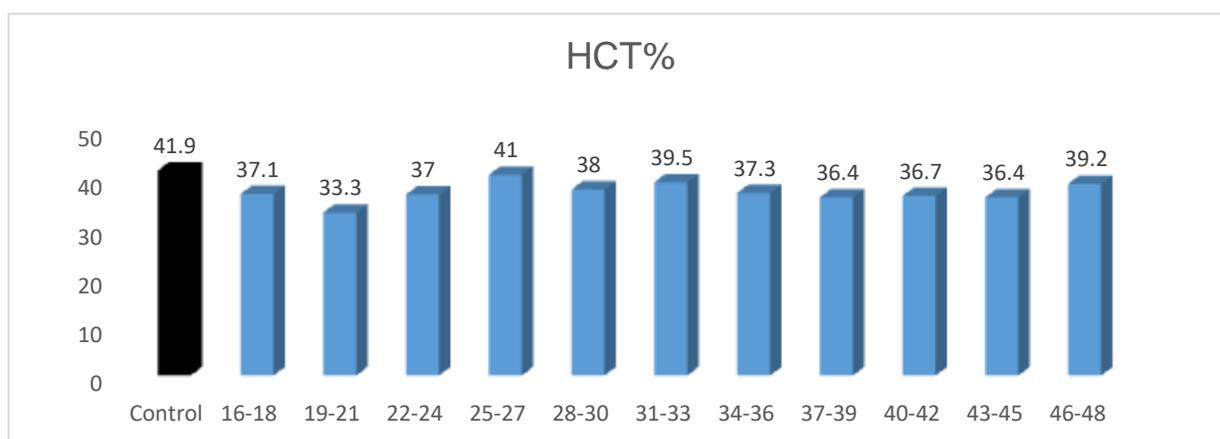


Fig.7: Various age groups (in years) and their Haematocrit percentage

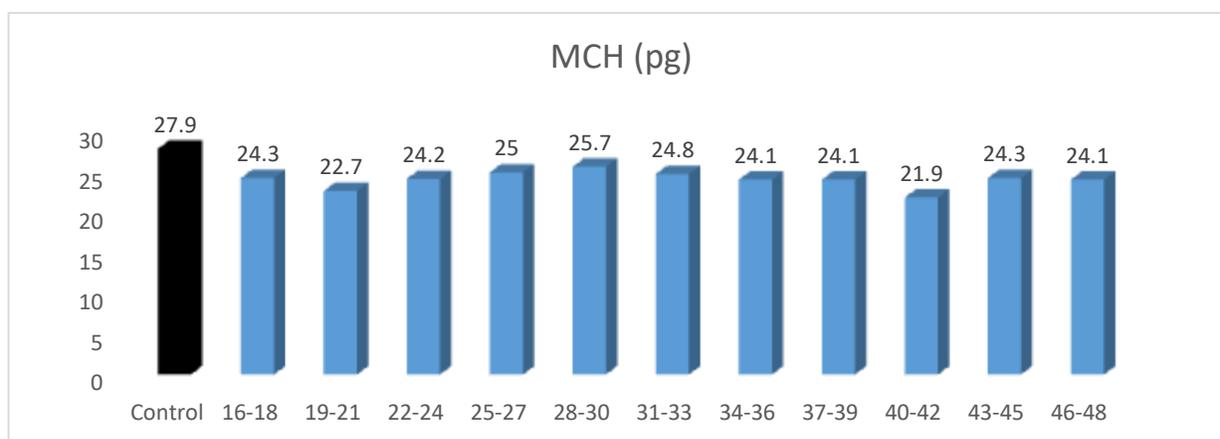


Fig.8: Various age groups (in years) and their mean corpuscular haemoglobin (MCH)

Roan mukharjee et al [12] said that the negative effects of smokeless tobacco and smoking tobacco had been almost very same.

Table 1: Hematological parameters of Gurhaku smokers

Parameters	Age (Study Group)											
	Control	16-18	19-21	22-24	25-27	28-30	31-33	34-36	37-39	40-42	43-45	46-48
RBC ($10^{12}/L$)	4.9	4.63	4.28	4.61	4.49	4.51	4.9	4.72	4.7	5.18	4.91	5.04
HGB (g/dL)	13.6	11.11	9.56	11.1	11.4	11.6	12.1	11.3	11.2	11.4	11.8	11.7
MCH (pg)	27.9	24.3	22.7	24.2	25	25.7	24.8	24.1	24.1	21.9	24.3	24.1
WBC ($10^9/L$)	8.2	7.5	8	7	8.7	7.9	8.2	8.5	9.1	10.6	12.3	12.8
Gran # ($10^9/L$)	4.9	4	5	3.8	4.3	8	4.1	5	5.4	5.7	6	6.5
Gran %	59.3	52.4	58.2	53.9	50.8	49.9	50.8	59	58.1	55.3	53	48.2
Lymph # ($10^9/L$)	2.3	2.6	2.6	2.4	3.4	2.8	3.1	2.5	2.7	3.3	5	4.8
Lymph %	29.4	36	32.1	34.4	40.6	37.6	37.8	29	30.5	32.3	32.3	36.4
HCT%	41.9	37.1	33.3	37	41	38	39.5	37.3	36.4	36.7	36.4	39.2
MCV (fL)	86.6	81.3	75.9	80.7	80.9	84.4	81.2	79.5	79.5	71.3	75.4	77.7
PLT ($10^9/L$)	288	355	337	306	380	335	321	307	308	332	397	367

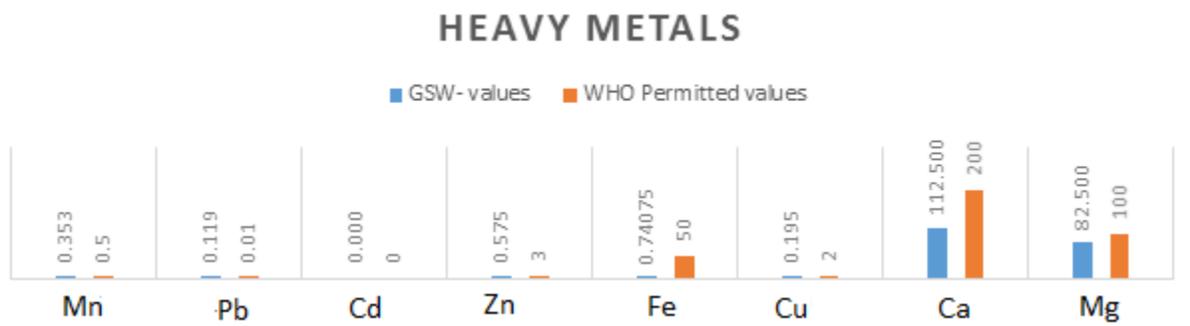
Elemental Analysis

The elemental analysis obtained by Atomic Absorption spectrometer revealed that the concentration (in ppm) of Mn, Pb, Cd, Zn, Fe, Cu, Ca and Mg were 0.353, 0.119, 0.000, 0.575, 0.741, 0.195, 112.500 and 82.500 respectively as shown in the table-2 below. The concentration of Pb found in the Gurhaku smoked water is higher than WHO permitted value which is 0.01 ppm and this finding is in agreement with the findings of

Akeel t al-kazwini et al [4] who found that the concentration of Pb was 0.037 in tobacco smoked Hookah water. The concentration of all other elements in the Gurhaku smoked water is lower than the permitted values of WHO and similar results were found by Akeel t al-kazwini et al in tobacco smoked water and reported the concentrations of Mn, Fe, Cu and Mg 0.035, 2.187, 0.046 and 37.931 respectively.

Table-2: Elemental analysis of Gurhaku smoked water (GSW) by using Atomic Absorption Spectroscopy and the values (in ppm) are mean of quadruplet.

Elements	Mn	Pb	Cd	Zn	Fe	Cu	Ca	Mg
GSW- values	0.35	0.12	0.00	0.57	0.74	0.20	112.50	82.50
WHO Permitted values	0.50	0.01	0.00-0.003	3.00	50.00	2.00	200.00	100.00

**Fig.9: Heavy metals concentrations (ppm) in Gurhaku smoked water (GSW) and WHO permitted values**

CONCLUSION:

The result of the current study revealed that the Ghurhaku smokers have slightly lower levels of Hb. They have significantly higher WBCs count including lymphocytes and granulocytes and slightly higher platelet count. However, RBC count, Mean Corpuscular volume (MCV), and Hematocrit percentage (HCT %) did not show any significant change. It is suggested based upon the results of the present study that hematological parameters in the patients of Ghurhaku smokers may be dealt in the hospitals with caution keeping in mind the disturbed Hb, WBC and platelet count including lymphocytes and granulocytes.

The elemental analysis of the current study revealed that the increased concentration of Pb may add into the soil pollution wherever the water of the Hookah is drained off.

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