



Mixed saliva of chemical industry workers in the context of X-ray fluorescence analysis

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Abstract

INTRODUCTION. Mixed saliva is an informative biological medium reflecting the general state of the body and sensitive to occupational exposure factors. Changes in its macro- and microelement composition under the influence of chemical agents can significantly affect the processes of mineralization and demineralization of dental hard tissues, as well as serve as an early diagnostic marker of dental and systemic pathologies.

AIM. To perform X-ray fluorescence analysis of mixed saliva in workers of chemical enterprises, assessing qualitative and quantitative changes in elemental composition and their relationship with clinical manifestations of dental pathology.

MATERIALS AND METHODS. A total of 121 workers of chemical industries with more than 5 years of occupational experience were examined. The control group consisted of 82 individuals not exposed to occupational hazards. Unstimulated saliva samples were collected in the fasting state. Elemental composition was determined using total reflection X-ray fluorescence analysis (TXRF) on an S2 PICOFOX spectrometer (Bruker) with selenium as an internal standard.

RESULTS. Significant deviations of the elemental composition of mixed saliva from physiological norms were revealed in workers of chemical enterprises. Increased concentrations of zinc, strontium, iron, and copper were observed along with decreased potassium levels. More than half of the examined individuals showed the presence of toxic elements, including lead and barium, as well as titanium. The levels of sulfur, chlorine, and manganese were generally within acceptable ranges. A decrease in phosphorus levels with relatively normal calcium content was established, accompanied by an increased Ca/P ratio, indicating the predominance of dephosphorylation processes. Clinically, this was associated with a high prevalence of non-carious lesions (erosion, wedge-shaped defects, increased tooth wear), accounting for approximately 65% of cases.

CONCLUSIONS. X-ray fluorescence analysis demonstrated pronounced disturbances in the elemental homeostasis of mixed saliva in workers of chemical enterprises, caused by occupational exposure. The identified changes, including accumulation of essential and toxic elements and imbalance of the calcium-phosphorus ratio, play a significant role in the development of predominantly non-carious dental lesions. These findings should be considered when developing preventive and therapeutic strategies, taking into account the specifics of the industrial environment.

Keywords: mixed saliva, X-ray fluorescence analysis, chemical industries, elemental homeostasis, Ca/P ratio, occupational exposure

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Смешанная слюна работников химических предприятий в свете рентгенофлуоресцентного анализа

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Резюме

ВВЕДЕНИЕ. Смешанная слюна является информативной биологической средой, отражающей общее состояние организма и чувствительной к воздействию профессиональных факторов. Изменение ее макро- и микроэлементного состава под влиянием химических агентов может оказывать существенное

влияние на процессы минерализации и деминерализации твердых тканей зубов, а также служить ранним диагностическим признаком развития стоматологической и соматической патологии.

ЦЕЛЬ. Провести рентгенофлуоресцентный анализ смешанной слюны работников химических предприятий с оценкой качественных и количественных изменений элементного состава и их связи с клиническими проявлениями стоматологической патологии.

МАТЕРИАЛЫ И МЕТОДЫ. Обследован 121 работник химических производств со стажем более 5 лет. Контрольную группу составили 82 человека без воздействия профессиональных вредностей. Сбор нестимулированной слюны проводили натощак. Элементный состав определяли методом рентгенофлуоресцентного анализа с полным внешним отражением (TXRF) на спектрометре S2 PICOFOX (Bruker) с использованием селена в качестве внутреннего стандарта.

РЕЗУЛЬТАТЫ. У работников химических предприятий выявлены значительные отклонения элементного состава смешанной слюны от физиологических норм. Отмечено повышение концентраций цинка, стронция, железа и меди при одновременном снижении калия. Более чем у половины обследованных обнаружено присутствие токсичных элементов, в том числе свинца и бария, а также титана. Содержание серы, хлора и марганца в большинстве случаев соответствовало допустимым значениям. Установлено снижение уровня фосфора при относительно нормальном содержании кальция, что сопровождалось увеличением индекса Ca/P и свидетельствовало о преобладании процессов дефосфорилирования. Клинически это проявлялось высокой частотой некариозных поражений (эрозии, клиновидные дефекты, повышенная стираемость), доля которых составила около 65% случаев.

ВЫВОДЫ. Методом рентгенофлуоресцентного анализа показано, что у работников химических предприятий происходит выраженное нарушение элементного гомеостаза смешанной слюны, обусловленное воздействием производственных факторов. Выявленные изменения, включая накопление эссенциальных и токсичных элементов и дисбаланс кальций-фосфорного соотношения, играют важную роль в развитии преимущественно некариозных поражений зубов. Полученные данные следует учитывать при разработке профилактических и лечебных мероприятий с учетом специфики производственной среды.

Ключевые слова: смешанная слюна, рентгенофлуоресцентный анализ, химические предприятия, элементный гомеостаз, индекс Ca/P, профессиональные вредности

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INTRODUCTION

Mixed saliva (MS) is widely regarded as a reflection of the body's physiological state and has been extensively studied for the early detection of pathological conditions and monitoring of metabolic responses to biologically active compounds, including those associated with occupational exposure [1–4]. Analysis of the macro- and microelement composition of MS is important both for understanding oral biological processes and for identifying electrolyte disturbances linked to systemic and local alterations. Detection of such imbalances may help clarify their causes, predict adverse physiological effects, and support timely preventive or corrective interventions.

Recent multidisciplinary studies combining chemical, biological, biochemical, and medical approaches have improved understanding of the complex processes occurring in the oral cavity with the involvement of MS. However, many aspects remain insufficiently understood. In particular, increasing attention has been focused on the role of essential and exogenous elements and their potential local and systemic effects [5; 6]. This includes their contribution to mineralization-demineralization processes, modulation of buffering capacity, and influence on metabolic,

redox, microbiome-related, and other physiological mechanisms.

Because MS is considered a sensitive biomarker of occupational exposure to chemical agents and anthropogenic environmental factors, its composition has been investigated using various physicochemical analytical techniques. Inductively coupled plasma mass spectrometry has been widely applied for elemental analysis [7; 8], together with photometric methods [9; 10] and biochemical assays [11]. The ionic composition of MS has also been assessed by capillary electrophoresis [12], while X-ray fluorescence analysis (XRF) has gained increasing attention in recent years [13–15].

Interest in XRF is largely related to its high analytical sensitivity and broad applicability in biological research. In studies of biological materials, XRF can provide valuable information about mechanisms involved in disease development and progression, potentially improving diagnostic and therapeutic approaches [16]. Recent advances in instrumentation have further expanded its analytical capabilities. Total reflection X-ray fluorescence (TXRF) enables elemental analysis without complex sample preparation. Unlike conventional XRF, TXRF uses monochromatic radiation and total

reflection geometry, which reduce signal scattering, matrix effects, and background noise. As a result, the method provides high sensitivity, including for trace elements. TXRF allows simultaneous multielement analysis of elements from aluminum to uranium, except niobium, molybdenum, and technetium.

AIM

The aim of this study was to investigate the qualitative and quantitative elemental composition of mixed saliva in employees of four chemical industry enterprises using total reflection X-ray fluorescence analysis (TXRF) and to evaluate possible associations between elemental alterations, pathological conditions, and potential long-term health effects. The findings may be useful for the development of primary dental preventive measures in occupational settings.

MATERIALS AND METHODS

The study was conducted at the Departments of Therapeutic Dentistry and Endodontics and General and Bioorganic Chemistry of the Russian University of Medicine (Moscow, Russian Federation), as well as at the laboratories of the Kurnakov Institute of General Inorganic Chemistry (Moscow, Russian Federation).

Dental status was evaluated in 121 employees and engineering personnel with at least 5 years of occupational exposure during the production of biologically active substances, paints and coatings, and metal products. All participants were routinely exposed to inorganic and organic chemical reagents in the workplace.

The control group included 82 dentally healthy individuals without metallic restorations or prosthetic appliances who were not taking medications or biologically active supplements and worked at the same enterprises in positions unrelated to chemical exposure.

Unstimulated MS samples were collected at the workplace in the morning under fasting conditions by expectoration into sterile sealed plastic tubes. Before sampling, participants were instructed not to brush their teeth or use mouth rinses to minimize contamination from oral hygiene products and reduce the risk of false-positive results.

Selenium (Se) was added to saliva samples as an internal standard. Several microliters of each sample were then applied onto a 30-mm quartz glass substrate using a micropipette, dried at 60°C for 12 h, and analyzed using a spectrometric system.

Elemental analysis was performed using an energy-dispersive total reflection X-ray fluorescence spectrometer S2 PICOFOX (Bruker AXS) according to ISO/TS 18705:2015 ("Surface chemical analysis – Use of total reflection X-ray fluorescence spectroscopy in biological and environmental analysis"). The average absolute sensitivity of the method was approximately 10⁻¹⁰%. Spectral lines of all detected elements were recorded simultaneously. The integrated software automatically performed spectral deconvolution, corrected for spectral overlap, background effects, and loss peaks, and calculated quantitative concentrations expressed in mg/L.

RESULTS AND DISCUSSION

The qualitative and quantitative composition of MS is influenced by multiple biological and environmental factors. Previous studies have shown that employees of chemical industry enterprises may exhibit either the presence of non-native elements in biological fluids or altered concentrations of essential elements.

No substantial deviations from physiological reference values reported in the literature were identified in the control group; therefore, these data are not presented separately.

Table 1 summarizes representative results of the elemental analysis of MS samples obtained from employees directly involved in industrial production and laboratory activities. The table mainly includes individuals with the most pronounced elemental alterations. Isolated findings of trace elements such as nickel (Ni), bromine (Br), and arsenic (As) were excluded because these cases were sporadic and showed no clear association with occupational exposure, although they are discussed later. Concentrations of biologically important macroelements, including calcium (Ca), phosphorus (P), and potassium (K), showed physiological variability within expected ranges.

The concentration of chloride (Cl) in unstimulated mixed saliva (MS) of healthy individuals is approximately 550 mg/L [17], although reported physiological values range from 180 to 2500 mg/L [18]. In the present study, Cl concentrations remained within physiological limits in nearly all samples. Slight reductions observed in several cases were unlikely to be clinically significant.

Most examined employees demonstrated elevated zinc (Zn) concentrations compared with reported physiological values [19; 20]. Increased salivary Zn promotes zinc phosphate deposition and may contribute to dental calculus formation [20]. Elevated Zn concentrations may also facilitate its incorporation into apatite structures of dental hard tissues. Patients No. 3 and 15, who demonstrated the highest Zn concentrations, presented with wedge-shaped cervical defects and mineralized dental plaque deposits, whereas patient No. 7 showed dental erosion. Previous XRF analysis of teeth obtained from employees of the same enterprises also confirmed Zn incorporation into dental hard tissues [21]. These findings suggest a possible role of Zn imbalance in enamel demineralization and non-carious dental lesions.

Strontium (Sr) was detected in all analyzed samples, and employees working in high-temperature industrial workshops demonstrated the highest concentrations (Table 1, patients No. 1, 3, and 15). Excessive Sr may replace calcium (Ca) in apatite structures and salivary gland acinar cells [7; 22; 23], potentially impairing mineralization processes. Employees with elevated Sr concentrations frequently demonstrated erosive lesions and wedge-shaped defects. Nickel (Ni), detected in two employees (patients No. 1 and 5), could also have contributed to these findings. Patient No. 1 additionally demonstrated elevated bromine (Br) concentrations. Altered Sr/Ba balance has been associated with cardiovascular and neurological disorders [24].

Therefore, pronounced deviations in salivary Sr and Ba concentrations may reflect broader systemic effects of chronic occupational exposure. Barium (Ba) concentrations were elevated in patients No. 1 and 15 and coincided with more severe dental pathology. Previous studies linked elevated salivary Ba concentrations to industrial environmental exposure [7; 22]. Increased Ba concentrations have also been reported in patients with Parkinson's disease [25], supporting the potential systemic relevance of this finding.

Potassium (K) concentrations were reduced in nearly all analyzed samples. Reduced salivary K may reflect autonomic dysregulation and chronic systemic imbalance associated with occupational exposure [26;27].

Titanium (Ti) was detected in more than 50% of analyzed samples, with the highest concentrations identified in patients No. 12 and 15. Ti exposure in this cohort was most likely associated with inhalation of titanium-containing industrial dust. Although the biological effects of chronic Ti exposure remain insufficiently understood, systemic distribution of Ti after pulmonary absorption may explain its presence in MS [28].

Elevated iron (Fe) concentrations were identified in all analyzed MS samples. In most cases, Fe concentrations exceeded reported physiological values several-fold [19; 20; 22; 29; 30]. Excessive Fe accumulation is

associated with oxidative stress and tissue damage [31]. Employees with elevated Fe concentrations frequently demonstrated erosive lesions and wedge-shaped defects and less commonly carious lesions. These findings may indicate chronic systemic effects associated with occupational exposure. Patient No. 11 additionally demonstrated detectable arsenic (As), although at a low concentration. Previous XRF analysis identified Fe incorporation into dental hard tissues only in isolated cases [21], suggesting limited incorporation of Fe into apatite crystal lattices despite elevated salivary concentrations.

Copper (Cu) concentrations substantially exceeded reported physiological values in nearly all analyzed samples [19; 20; 22; 30; 32]. Elevated Cu concentrations have been associated with impaired osteoblast viability, increased gingival permeability, and progression of periodontal disease and dental caries [33]. In the present study, elevated Cu concentrations were associated with multiple erosive and carious lesions, particularly in patients No. 1 and 15. Previous investigations in employees of the same enterprises did not demonstrate Cu incorporation into dental hard tissues [21], indicating selective incorporation of trace elements into apatite structures. Elevated salivary Cu concentrations have also been reported in oral precancerous lesions and oral cancer [34; 35].

Table 1. Elemental composition in mixed saliva of surveyed chemical industry workers (mg/L)

Таблица 1. Содержание элементов в смешанной слюне обследованных работников химических предприятий (мг/л)

Patient	Cl	Zn	Sr	K	Ti	Ba	Fe	S	Cu	Pb	Mn	Ca	P	Ca/P
Metallurgical enterprise														
1	265.27	–	2.784	403.96	–	38.250	6.376	23.42	0.355	0.219	0.061	37.40	36.73	1.02
2	148.26	1.203	0.142	200.00	0.048	–	3.062	20.46	0.087	–	–	40.00	14.23	2.81
3	384.90	4.004	5.165	400.00	–	–	13.435	25.13	–	0.150	–	53.07	42.56	1.25
4	179.30	3.070	0.117	177.60	0.0048	0.012	1.730	13.06	0.101	0.020	–	17.05	22.17	0.77
5	172.40	1.105	0.107	139.40	–	0.011	6.320	17.33	0.173	–	0.022	35.80	7.54	4.75
Chemical-pharmaceutical enterprise														
6	203.23	0.771	0.153	184.65	0.306	0.082	9.970	21.53	0.061	0.010	0.036	36.22	21.74	1.67
7	196.40	4.250	0.095	193.50	–	0.020	4.820	23.16	0.048	0.074	0.054	21.80	9.95	2.19
8	98.85	0.997	0.238	150.00	0.014	0.024	2.830	24.07	0.136	–	0.021	37.30	9.56	3.90
9	92.87	0.499	0.275	134.10	–	–	2.634	23.34	0.116	–	–	24.94	18.13	1.38
Agrobiological enterprise														
10	183.70	1.224	0.084	161.30	–	–	4.190	11.28	0.097	0.009	0.030	40.05	11.31	3.54
11	256.80	2.122	0.134	141.40	–	–	1.080	5.84	0.007	0.003	–	51.60	34.21	1.51
12	216.95	1.401	0.258	312.94	2.007	0.367	4.386	42.49	0.134	0.470	–	30.89	54.27	0.57
13	95.89	0.818	0.143	164.36	0.053	0.082	2.300	8.07	0.065	–	–	18.80	12.22	1.54
Enterprise manufacturing paints, coatings, and anticorrosion materials														
14	64.83	0.238	0.087	98.72	0.021	0.013	1.520	3.57	0.003	–	–	12.45	7.12	1.75
15	153.35	8.863	8.774	227.50	0.950	1.016	10.862	23.79	0.263	1.082	–	101.33	41.88	2.42

Lead (Pb) was detected in more than half of the examined employees, and in several cases, concentrations exceeded permissible levels by one or two orders of magnitude. The highest concentrations were identified in employees working in high-temperature industrial workshops, suggesting inhalational exposure. Pb accumulation is known to disrupt redox balance and interfere with mineral metabolism [36]. Employees with elevated Pb concentrations predominantly demonstrated non-inflammatory dental lesions, including erosion, pathological tooth wear, and wedge-shaped defects.

Manganese (Mn) was detected in fewer than half of analyzed samples at concentrations generally corresponding to reported physiological values [7; 19; 20; 22]. Previous XRF analysis identified Mn incorporation into dental hard tissues only in isolated cases [21]. However, the chemical forms of Mn were not determined, limiting interpretation of its biological effects.

Sulfur (S) concentrations remained within physiological limits in all analyzed samples despite pronounced imbalance of other elements. Although salivary sulfur composition has been associated with periodontal status [37–39], occupational elemental exposure in the present cohort likely reduced the diagnostic significance of S fluctuations.

Calcium (Ca) concentrations were generally within physiological limits but close to the lower boundary of normal values, whereas phosphorus (P) concentrations were reduced in nearly all samples. Reduced phosphate availability may enhance demineralization processes by promoting phosphate migration from

dental hard tissues and increasing the Ca/P ratio. Our previous study demonstrated that this pattern is more characteristic of non-inflammatory dental hard tissue lesions than dental caries [16]. In the present study, the Ca/P ratio exceeded physiological values in most samples. Clinical examination of employees of chemical enterprises revealed approximately twice as many cases of dental erosion, wedge-shaped defects, and pathological tooth wear as carious lesions, consistent with our previous observations.

CONCLUSION

Total reflection X-ray fluorescence (TXRF) analysis of mixed saliva from employees of four chemical enterprises demonstrated deviations in elemental composition consistent with occupational exposure:

1. Increased concentrations of Zn, Sr, Fe, and Cu were observed, alongside reduced salivary K levels, indicating altered systemic mineral homeostasis.

2. Saliva samples contained exogenous elements, including Pb, Ba, and Ti, reflecting environmental and occupational contamination.

3. S, Cl, and Mn were detected within ranges that did not indicate toxicological concern under the studied conditions.

4. Elevated Ca/P ratios suggest a shift toward dephosphorylation-dominant processes. Clinically, non-inflammatory dental lesions prevailed (65%) over carious lesions (35%) in the examined cohort.

The findings should be considered when developing preventive and therapeutic strategies, taking into account the specifics of the industrial environment.

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