

## Effects of Exercise, Games, and Dance on Trace Element Concentrations in Human Saliva

Manisha Yadav<sup>#</sup>, Jyoti Yadav<sup>§</sup> and Arup Giri<sup>#,\*</sup>

<sup>#</sup>Department of Zoology, Baba Mastnath University, Rohtak - 124 021, India

<sup>§</sup>Department of Zoology, Maharshi Dayanand University, Rohtak - 124 001, India

\*Email: arupsatadal@gmail.com

### ABSTRACT

Physical activities and sports are an essential part of our life. The interest of the younger generation in physical health is rapidly increasing. Therefore, there is also a need to identify and correct sport-linked misbalances and mineral imbalances. Saliva is an easy-to-collect, harmless biological fluid that might help us. Exercise is known to affect the trace elements level in biological substances. We aimed to highlight the significance of saliva as a mineral status biomarker. Sixty-eight healthy subjects participated in the study. Twenty-nine participants performed exercises, 26 played cricket games, and 13 participated in dance activities. Saliva samples were taken just before and after all three activities. Sixteen elements were analysed through inductively coupled plasma–mass spectrometry (ICP–MS). Among the 16 elements, four, namely, sodium (Na), boron (B), potassium (K), and iron (Fe), significantly varied. A substantial increase in trace element concentrations in saliva ( $p < 0.05$ ) was observed after games (Fe), dances (Na, K, and Fe), and exercise activities ( $p < 0.05$ ) (B, Na, and K). A significant decrease ( $p < 0.05$ ) in the trace element concentration in saliva was observed after cricket (B) activity, and a significant reduction ( $p < 0.05$ ) in the Fe concentration was observed after exercise activity. Our study highlights the use of saliva as a potential biomarker for mineral status evaluation. Saliva might be a beneficial tool for correcting mineral-associated imbalances in sports. If we successfully identify mineral deficiency through saliva, then there are also chances to diagnose mineral-based deficiency diseases at an early stage. To achieve this goal, further detailed research is needed. More research with a large population size is needed, considering the effects of changes in the intensity and regularity of physical activities on saliva composition and the associations of trace elements with health conditions.

**Keywords:** Biomarker; Health; Inductively coupled plasma–mass spectrometry; Mineral status; Physical activities; Saliva composition

### 1. INTRODUCTION

Saliva is a sound biological sample for medical research because it contains various compounds, such as vitamin C, malondialdehyde, amylase, and proteomes. Saliva collection is an effortless process and requires little training. Saliva can be used as a biospecimen to reflect an individual's health status. Researchers are investigating the possible connection between oxidative stress and its negative effects. Saliva serves as a source of systemic biomarkers that reflect oxidative stress. However, it is still being determined whether saliva is as reliable as blood and plasma for this purpose<sup>1</sup>. Whole saliva is a composite mixture of epithelial cells, saliva, food debris, gingival crevicular fluid, and microorganisms. A healthy individual produces 500–1500 mL of whole saliva at an average speed of 0.3–0.4 mL/min.

Saliva composition is dynamic and influenced by different stimuli<sup>2,3</sup>. Saliva is a complex fluid derived from various local and systemic sources, giving it the potential to indicate both systemic and oral health conditions<sup>4</sup>. Salivary compositional changes occur during different physiological conditions. Saliva secretion depends on nerve-mediated stimuli, which can be influenced by factors such as food intake, aging, and disease<sup>5</sup>. Many studies have explored the correlation between salivary biological properties and physical exercise. Changes in saliva composition during and after exercise highlight the significance of physical activity, its potential risks, and the role of saliva as a health indicator. Saliva analysis can be used to measure exercise-induced changes, including lactate accumulation, hormonal adaptations, and variations in immunological markers. Salivary enzymes, hormones, and immunoglobulins can be affected by variations in physical condition, the nature and frequency of training, and the overall

health of athletes. Therefore, further investigations considering the correlation between physical exercise and saliva, especially salivary organic components, are needed. The available knowledge<sup>6</sup> must benefit sports activities<sup>6</sup>. It has also been reported that dance affects saliva composition. According to one study, evening cortisol in saliva decreased after eight months of dance and yoga intervention among girls with functional abdominal pain disorders (FAPDs)<sup>7</sup>. Salivary parameters such as hormones, immunoglobulins, and enzymes are also affected by intense physical stress, such as ultramarathon running<sup>8</sup>. Various studies have shown that a close association between systemic diseases (obesity, cardiovascular diseases, type 2 diabetes, and metabolic syndrome) and chronic inflammation, oxidative stress, and serum levels of metal ions has been acknowledged through earlier investigations<sup>9-12</sup>. This is why we selected trace element concentrations in saliva for our study. Any disturbance in the homeostasis of trace elements may lead to the expansion of pathologic states and diseases in trace elements<sup>13</sup>. These are of two types: essential and nonessential trace elements. The important features include Fe, Cu, Co, I, Mn, and Zn, and the nonessential trace elements are Ag, Cd, Hg, and Pb<sup>14</sup>. Saliva also contains a range of essential minerals that regulate oxidative stress, inflammatory cytokine marker levels, and protection from periodontal tissue damage<sup>15,16</sup>. For the regulation of the structural integrity of connective and epithelial cells, significant elements such as potassium (K) and sodium (Na) are involved<sup>17</sup>, and calcium (Ca), rubidium (Rb), magnesium (Mg), and iron (Fe) are the elements that are recognised to be involved in periodontal ligament homeostasis and alveolar bone remodelling modulation;<sup>15,18,19</sup> similarly, lithium (Li) is involved in bone regeneration<sup>20</sup>.

Sports are currently a very positive trend for public health, and the interest of the younger generation in recreational sports is increasing. Therefore, saliva is also necessary to identify and correct sport-linked imbalances and mineral imbalances, and the painless biological fluid that might help us do so is saliva<sup>21</sup>. Saliva is essential because it contains viruses, metabolic products, immunological products, biomarkers, and hormones that might help to evaluate, diagnose, and prevent systemic health issues<sup>22</sup>. There are more reasons to select saliva in our experiment. Trace element evaluation has shown promising results in the diagnosis of oral submucous fibrosis<sup>23</sup>, and saliva is a potential noninvasive method for monitoring health and the instantaneous diagnosis of multiple diseases in real time<sup>24</sup>. Saliva has also been used in forensic odontology and as a diagnostic tool with trace element differentiation in nondiabetic and diabetic individuals<sup>25,26</sup>. All these studies showed that changes in different health ailments affect the trace element concentrations in saliva.

For the first time, this study examines changes in the trace element composition of saliva before

and after three physical activities-sports, dance, and exercise-to emphasise both the benefits of various physical activities and the potential of trace elements as biomarkers for detecting multiple health conditions and deficiency diseases at an early stage. The oral microbiota is increasingly recognised as a key factor influencing host physiology and disease states. However, the factors affecting oral microbiota composition remain insufficiently characterised, necessitating further population-based studies<sup>27</sup>. Despite growing knowledge about the role of metal ions in periodontal health<sup>28-30</sup>, there is still a need for more research on changes in the salivary ionic profile during periodontal disease. Additionally, limited studies have explored how different activities, such as dance, sports, and exercise, influence the mineral composition of human saliva. This study highlights the impact of physical activities-including exercise, dance, and sports-on trace element concentrations in saliva and underscores their significant role in exercise physiology.

## 2. METHODOLOGY

The research was conducted at selected study sites, including Rework Fitness Gym, Maharshi Dayanand University Cricket Stadium, and Vibe Dance Studio in Rohtak, Haryana, India. A total of 78 participants were initially enrolled in the study, with 10 being excluded. Individuals with systemic diseases, smoking habits, diabetes, or oral health issues were not included in the research. All participants were above 18 years of age. The final study group comprised 68 healthy individuals, with 29 engaging in gymnasium exercise, 26 participating in cricket, and 13 involved in dance activities. The duration of the dance session was 45 minutes, the cricket activity lasted 3 hours, and the exercise session was 1 hour. Saliva samples (3-5 mL) were collected twice, both before and after the respective activities (Fig. 1).

### 2.1 Ethical Clearance

All the participants signed a consent letter and voluntarily participated in the study. The subjects also filled out a questionnaire mentioning their health status. All the volunteers were well instructed in this experimental study before the commencement of the experiment. Therefore, there was no requirement for ethical clearance.

### 2.2 Sample Collection Method

For the spitting method, saliva was collected in 15 mL Falcon tubes. The participants were asked to spit saliva in the falcon tubes before and after physical activity<sup>31</sup> (Fig. 2).

### 2.3 ICP-MS Analysis

The elements that were selected for ICP-MS measurement were manganese (Mn), zinc (Zn), cobalt (Co), chromium (Cr), iron (Fe), silver (Ag), copper

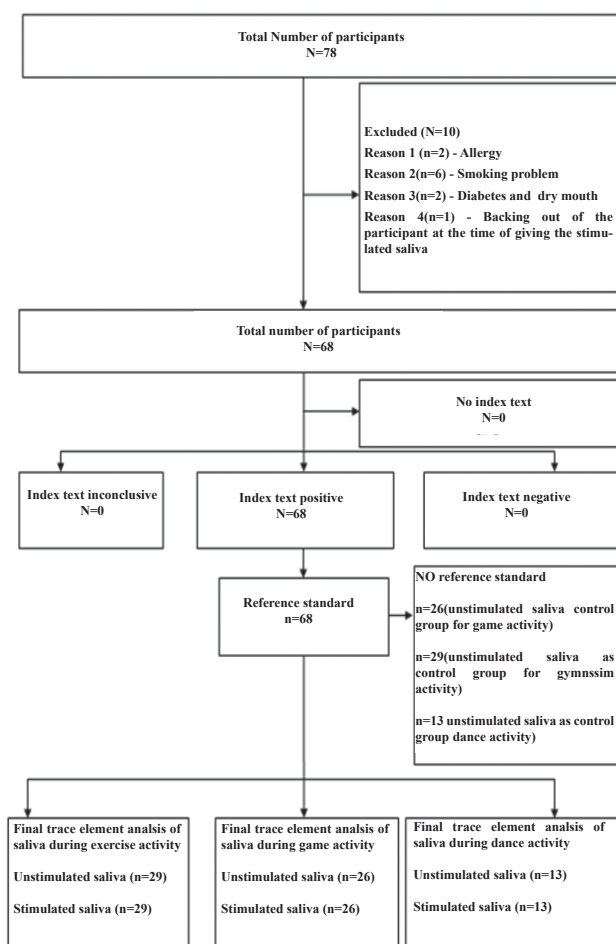


Figure 1. Prototypical STARD diagram to report the course of participants throughout the research.

(Cu), cadmium (Cd), nickel (Ni), magnesium (Mg), potassium (K), lead (Pb), boron (B), aluminum (Al), sodium (Na), and calcium (Ca). The following methodology was used to analyse minerals in saliva. The pooled saliva was subsampled after centrifugation at  $4000 \times g$  for 15 min. Three-millilitre aliquots were diluted with 4 mL of tertiary distilled water and 4 mL of 70 % ultrapure nitric acid for microwave-assisted acid digestion. Afterward, acid digestion was performed in a microwave for 20 min (180 °C, 200 psi). Hereafter, with tertiary distilled water, the aliquots were diluted 16.7-fold<sup>32</sup>. To ensure the resolution of spectral interferences, sensitivity, isotope selection, and mass resolution were optimised for each element. Following a 60 s uptake and stabilisation period, each sample was analysed. The nebulizer system was rinsed for 1 min with 2 % subboiled  $\text{HNO}_3$  between samples, eradicating carry-over and tuning the sampler cone. The whole system was provided with a 1270 W power function. For the auxiliary and nebulizer gases, a flow rate of 1 L/min was used, and the plasma gas flow rate was 16 L/min. Three times the standard deviation of the blank samples was taken as the limit of detection (LOD) for each element, and the elements were defined by Romano<sup>33</sup>, *et al.*

## 2.4 Statistical Analysis

An independent t test was performed on the datasets of all three activities via Statistical Packages for Social Sciences (SPSS) software to identify possible significant differences between the groups. The significance level was set at  $p < 0.05$ .

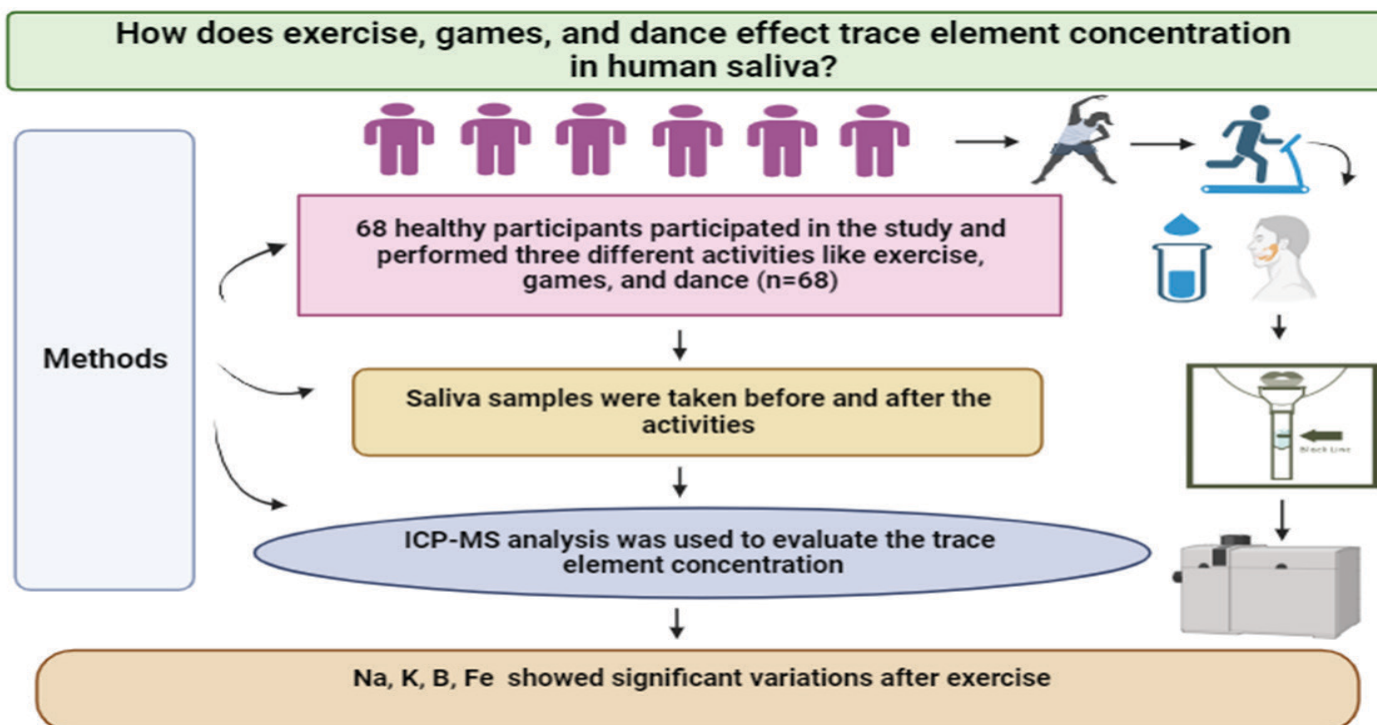


Figure 2. Overall view of the effects of exercise, dance, and games on trace element concentrations in human saliva.

### 3. RESULTS

A significant decrease ( $p < 0.05$ ) in B levels was detected after cricketing activity compared with before activity, and a significant increase ( $p < 0.05$ ) in Fe concentration was detected after game activity (Fig. 3B and 3C). Before cricket activity, the B concentration was  $3.12 \pm 0.38$  ppb. After cricket activity, the B concentration was  $2.62 \pm 0.07$  ppb (Fig. 3A). Before cricket activity, the Fe concentration was  $9.40 \pm 0.16$  ppb, and after cricket activity, the Fe concentration was  $11.19 \pm 2.22$  ppb (Fig. 3C).

A significant increase ( $p < 0.05$ ) in the Fe, Na, and K concentrations was detected after dance activity (Fig. 4C and 4D). Before the dance activity, the Na concentration was  $45.17 \pm 1.51$  ppb, the K concentration was  $31.70 \pm 0.90$  ppb, the Fe concentration was  $7.85 \pm 0.05$  ppb, and after the dance activity, the Na concentration was  $106.14$

$\pm 46.85$  ppb, the K concentration was  $193.87 \pm 153.78$  ppb, and the Fe concentration was  $8.47 \pm 0.22$  ppb (Fig. 4C and 4D).

A significant decrease ( $p < 0.05$ ) in Fe levels was detected after exercise activity compared with before exercise activity, and a substantial increase ( $p < 0.05$ ) in B, Na, and K concentrations was detected after exercise activity (Fig. 5A and 5B). Before the exercise activity, the concentration of Fe was  $11.72 \pm 0.08$  ppb. After exercise, the Fe concentration was  $8.56 \pm 0.20$  ppb (Fig. 5D). Before exercise activity, the Na concentration was  $44.47 \pm 3.86$  ppb, the K concentration was  $31.70 \pm 0.72$  ppb, the B concentration was  $1.72 \pm 0.08$  ppb, and after exercise activity, the Na concentration was  $244.60 \pm 174.96$  ppb, the K concentration was  $909.93 \pm 846.23$  ppb, and the B concentration was  $2.49 \pm 0.68$  ppb (Fig. 5A and 5D).

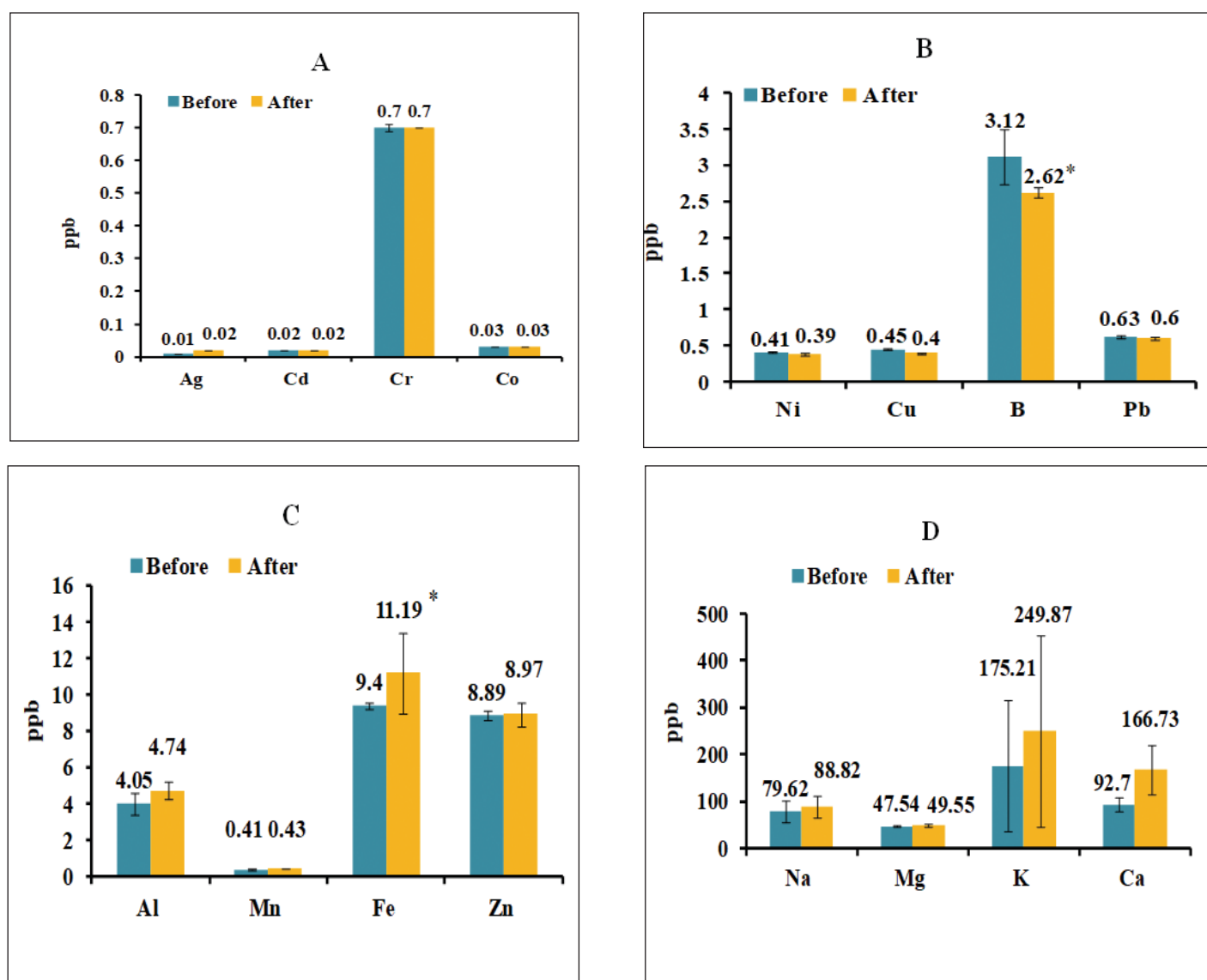


Figure 3. Graph showing the concentrations of heavy metals in saliva during game activity (A, B, C & D represent all 16 elements: manganese (Mn), zinc (Zn), cobalt (Co), chromium (Cr), iron (Fe), silver (Ag), copper (Cu), cadmium (Cd), nickel (Ni), magnesium (Mg), potassium (K), lead (Pb), boron (B), aluminum (Al), sodium (Na), and calcium (Ca). (\*) represents  $p < 0.05$ .

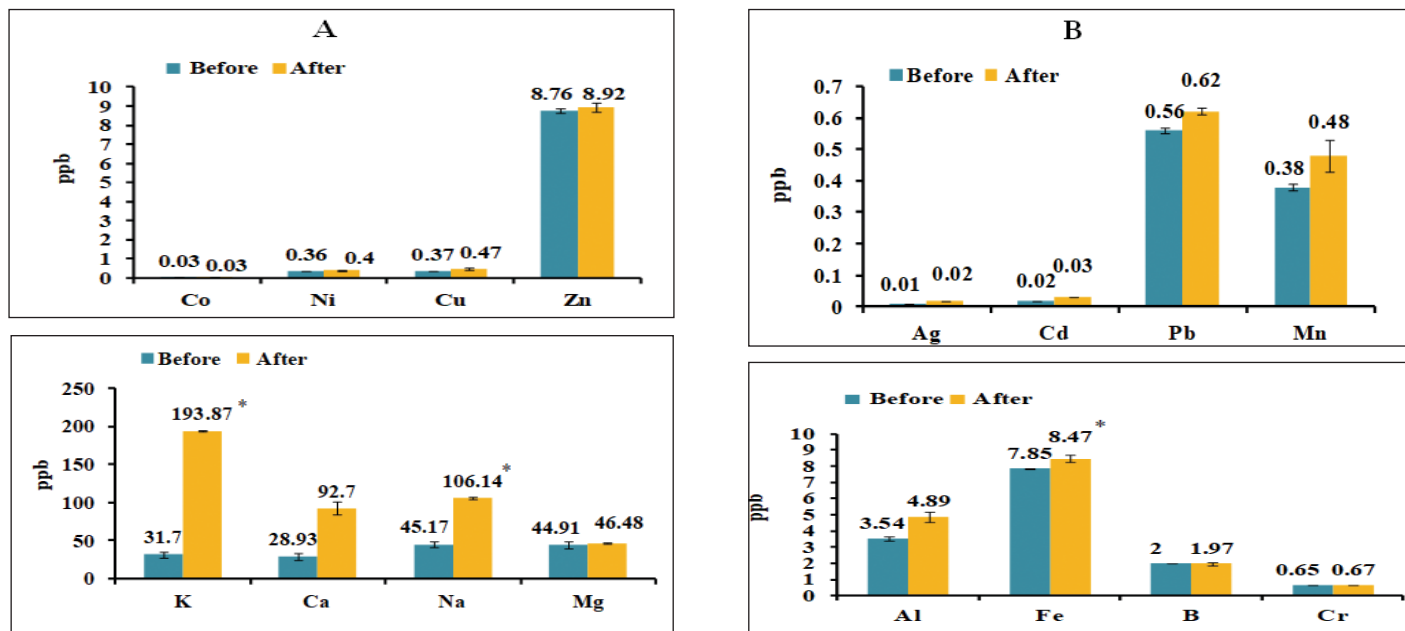


Figure 4. Graph showing the concentrations of heavy metals in saliva during dance activity (A, B, C, & D represent all 16 elements: manganese (Mn), zinc (Zn), cobalt (Co), chromium (Cr), iron (Fe), silver (Ag), copper (Cu), cadmium (Cd), nickel (Ni), magnesium (Mg), potassium (K), lead (Pb), boron (B), aluminum (Al), sodium (Na), and calcium (Ca). (\*) represents  $p < 0.05$ .

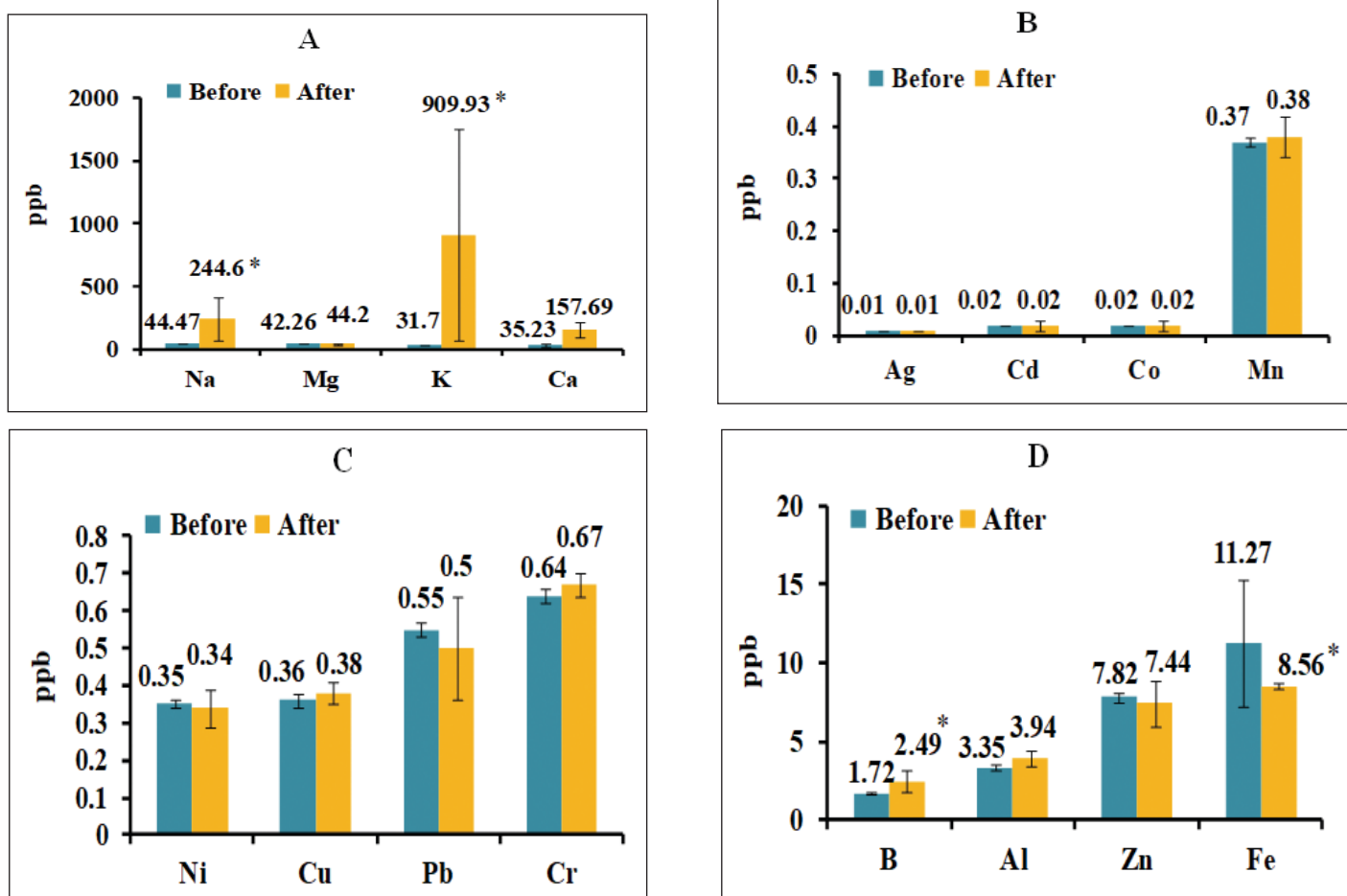


Figure 5. Graph showing the concentrations of heavy metals in the saliva during gymnasium exercise activity (A, B, C, & D represent all 16 elements: manganese (Mn), zinc (Zn), cobalt (Co), chromium (Cr), iron (Fe), silver (Ag), copper (Cu), cadmium (Cd), nickel (Ni), magnesium (Mg), potassium (K), lead (Pb), boron (B), aluminum (Al), sodium (Na), and calcium (Ca). (\*) represents  $p < 0.05$ .



#### 4. DISCUSSION

A significant increase in trace element concentrations in saliva ( $p < 0.05$ ) was observed after games (Fe) (Fig. 2C), dances (Na, K, and Fe) (Fig. 4C and 4D), and exercise activities (B, Na, and K) (Fig. 5A and 5D). The increase in Na and K concentrations after exercise and dance activities might increase sodium and potassium concentrations after physical activity because adrenal sympathetic activity at high exercise intensities might lead to an increase in salivary electrolyte concentrations. However, the sodium and potassium concentrations in saliva increase after physical activity, but the exact mechanism in saliva still needs to be mentioned in the abstracts available<sup>34,6</sup>.

Our research revealed that the Fe concentration increased after the participants played cricket games (Fig. 3C). This could be due to long-term physical training that affects homeostasis and iron metabolism. According to a study on soccer players, plasma and serum iron concentrations were higher than those in the untrained control group. These findings suggest that physical training affects iron distribution within the body, with more iron available in the extracellular compartment. However, further analysis is needed to assess overall iron status, as iron concentrations can vary between different body compartments<sup>35</sup>. The Fe concentration after dance was reportedly high (Fig. 4H), possibly because of physical activity, which might have affected the salivary nitrate and nitrite levels. Exercise can also affect the salivary flow rate, which might cause the Fe concentration to increase<sup>36,37</sup>. A significant decrease ( $p < 0.05$ ) in the B trace element concentration in saliva was observed after cricketing and exercise activity (Fig. 3B, Fig. 4D, and Fig. 5D), and a significant decrease ( $p < 0.05$ ) in the Fe level was detected after exercise activity (Fig. 5D).

The reason for the decrease in the B concentration compared with those of the other elements might be related to the respiration rate. A higher respiration rate during physical activity might cause boron removal from the body via exhaled air<sup>38</sup>. Moreover, the B concentration was greater in saliva after exercise. After gymnasium exercise, the relatively high B concentration in the saliva might be attributed to several physiological mechanisms. The mechanical stress of exercise may enhance bone remodelling and mobilize boron into the bloodstream, leading to increased saliva levels. A higher rate of blood flow and improved perfusion of salivary glands during physical activity also facilitate the transport of B to the glands, resulting in greater salivary secretion. Moreover, the altered pH and ionic composition of saliva during physical exertion might increase B solubility and secretion. Therefore, the increased metabolic activity during exercise might mobilize B as part of enzymatic and metabolic pathways, contributing to its increased excretion in saliva. Jointly, these mechanisms suggest a multifaceted interplay of physiological changes leading to elevated B levels in saliva postexercise<sup>39</sup>. However, the Fe concentration decreases after exercise due to

several factors. One possible reason for the decreased Fe concentration after exercise is increased iron regulatory hormone hepcidin, which inhibits dietary iron absorption<sup>40</sup>. Another reason for the decrease in Fe concentration is inflammation, which results in biochemical changes in the erythrocyte cell membrane because of oxidative stress caused by exercise, which causes damage and increases intravascular hemolysis<sup>41</sup>.

#### 5. CONCLUSION

It has been concluded from the present study that out of 16 elements, four significantly changed in saliva composition after different physical activities. The aspects that showed considerable changes were Na, B, K, and Fe. The primary purpose of this study was to reflect the importance of saliva as a valuable indicator of mineral imbalances caused by high body mineral demand during physical activities. Saliva might be a beneficial tool for correcting mineral-associated imbalances in sports. We observed trace element changes in saliva composition after exercise, dance, and game activities, and these changes may be helpful for further clinical implications. Our study highlights the use of saliva as a potential biomarker for mineral status evaluation. Saliva is easy to collect, store, handle, and convenient for use as a biofluid. Therefore, more research is needed on the effects of physical activity frequency and intensity on the salivary composition of a large population, which would be helpful for researchers in further clinical evaluations highlighting salivary biomarkers.

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## CONTRIBUTORS

**Mrs Manisha Yadav** has completed her Masters degree in Zoology from Maharshi Dayanand University, Rohtak. She is currently perusing her PhD work in Baba Mastnath University, Rohtak, Haryana. Her research area is human salivary biomarkers and its correlation with human physiology. She collected all the samples, accomplished all the analysis work, and prepared primary draft.

**Mrs Jyoti Yadav** has completed her Masters degree in Zoology from Maharshi Dayanand University (MDU), Rohtak, Haryana. She is currently pursuing her PhD work in Maharshi Dayanand University, Rohtak, Haryana. Her research area is molecular biology and its correlation with human physiology. She helped first author during sample collection and sample analysis.

**Dr Arup Giri** is currently working as Associate Professor in Department of Zoology and Director of Internal Quality Assurance Cell (IQAC), Baba Mastnath University, Rohtak, Haryana. Earlier, he completed his PhD in Life Sciences from DRDO-Defence Institute of High Altitude Research (DIHAR), Ladakh, India. His area of research expertise is environmental toxicology and animal physiology. He conceived, supervised, revised and edited the final manuscript.