

# Determination of Water Quality Indices and Assessment of Heavy Metal Pollution of Drinking Water Sources in Thimphu

Chimmi Dorji<sup>1</sup>, Pema Chophel<sup>2</sup>

<sup>1-2</sup>Royal Centre for Disease Control, Ministry of Health, Serbithang, Thimphu, Bhutan

## ABSTRACT

**Introduction:** The well-being of public health is associated with the availability of safe and clean drinking water. Heavy metals, known for their toxicity and potential health risks, are a particular focus of water quality assessments. This study investigates the heavy metal concentrations in five primary drinking water sources of Thimphu city: Motithang, Jungshina, Taba, Dechencholing and Chamgang. **Methods:** The ten heavy metals, known for their toxicity (Aluminum (Al), Boron (B), Barium (Ba), Cadmium (Cd), Chromium (Cr), Copper (Cu), Manganese (Mn), Nickel (Ni), Lead (Pb), and Zinc (Zn)), were assessed using inductively coupled plasma optical emission spectroscopy (ICP-OES) for precise analysis. The study employed the Heavy Metal Pollution Index (HPI) and the Heavy Metal Evaluation Index (HEI) to evaluate water quality. **Results:** The test results revealed low heavy metals concentration in the water sources. The HPI and HEI values were also calculated to be low (<15 and <1.24 respectively). **Conclusions:** The study provides valuable insights into Thimphu's drinking water quality, indicating a positive status with low heavy metal concentrations.

Keywords: Drinking water; Heavy metals; Pollution index; Public health; Water quality.

#### INTRODUCTION

Access to safe and clean drinking water is a fundamental necessity for safeguarding public health<sup>1</sup>. Contamination of water sources by heavy metals poses a significant threat due to their wellestablished toxicity and potential adverse health effects. Exposure to these metals may lead to adverse health effects such as cancer, hypertension, lung disease, gastrointestinal bleeding, renal disease and reproductive effects<sup>2-4</sup>. With the evident carcinogenic effects and toxic nature of heavy metals, combined with their persistent bioavailability in water, regulatory bodies such as World Health Organization (WHO) and United States Environment Protection Agency (USEPA) have proposed the Maximum Permissible Limits (MPL) in drinking water<sup>5,6</sup>. Additionally, Bhutan has established its own drinking water quality standard, the Bhutan Drinking Water Quality Standard (BDWQS) in 2016, outlining the recommended maximum permissible limits for specific heavy metals in drinking water<sup>7</sup>.

Globally, there are millions of people with chronic heavy metal poisoning out of which 1.6 million children alone die each year from diseases attributed to contaminated drinking water making it a public health concern<sup>8</sup>. The presence of heavy metals in water sources can be attributed to both natural occurrences, such as eroded minerals form rocks, leaching of ore deposits, and volcanic activities, as well as anthropogenic activities like improper solid waste disposal and industrial or domestic effluents<sup>9</sup>. While Bhutan is renowned for its pristine environment and clean water resources, the escalating population and increased human activities in various regions have raised concerns about the quality of drinking water, particularly in areas undergoing rapid urban development and growth. Furthermore, in Bhutan, the existing studies that assess water quality and pollution sources, arising from both natural processes and human activities, are currently limited.

Thimphu, the capital of Bhutan, is home to the country's largest population, with around 138,736 residents, constituting 19.1% of the nation's total population<sup>10</sup>. National projections indicate that by 2047, Thimphu is expected to accommodate nearly 30% of Bhutan's entire population<sup>11</sup>. With increasing developmental activities, the city's water sources confront the constant risk of contamination, posing a significant public health threat. Given that the primary drinking water sources in Thimphu mainly consist of streams and rivers, continuous monitoring of these sources, along with comprehensive assessments of heavy metals, is crucial to ensure the quality of drinking water and sustainable resource management.

This article discusses a comprehensive investigation focusing on the presence and concentrations of ten critical heavy metals, namely Aluminum (Al), Boron (B), Barium (Ba),

**Corresponding author:** 

Chimmi Dorji cdorji@health.gov.bt

Cadmium (Cd), Chromium (Cr), Copper (Cu), Manganese (Mn), Nickel (Ni), Lead (Pb), and Zinc (Zn), in the drinking water sources of Thimphu. The primary objective of this study is to evaluate the drinking water for level of concentration of heavy metals, specifically addressing heavy metal contamination in Thimphu. To achieve this, water samples from five primary sources were analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES). Further, to assess the extent of heavy metal pollution in drinking water sources, the heavy metal pollution index (HPI) and heavy metal evaluation index (HEI) are used<sup>12,13</sup>. These indices provide a comprehensive understanding of the overall water quality based on multiple parameters<sup>14</sup>. Consequently, this research aims to provide valuable insights for environmental managers, policymakers, and decision-makers, facilitating strategic interventions to ensure the provision of safe drinking water for Thimphu's residents. It also emphasizes the broader significance of water quality monitoring in similar regions around the world.

## METHODS

#### Study Area

Thimphu city, nestled in western Bhutan, is demarcated by its geographical coordinates of latitude 27° 28' 22.0512" N and Longitude 89° 38' 21.4296" E. Situated at an altitude of approximately 2,320 meters above sea level, the city occupies an area of approximately 1,840 square kilometers. The city is surrounded by picturesque mountains and valleys, and its proximity to the Wang Chuu River enhances its geographic beauty.

The Wang Chuu River system plays a vital role in Thimphu's landscape, providing not only a source of drinking water but also contributing to the city's aesthetic charm. Alongside the Wang Chuu River, numerous smaller streams converge, contributing to the primary water source for drinking purposes. Figure 1 shows the location of the various water sampling points in Thimphu.

#### Water Sample Collection



Figure 1. Location and sampling points in Thimphu City, Bhutan

Water samples were systematically obtained from the raw water intake points (in March 2023) of the drinking water treatment plants (WTP) situated at Motithang, Jungshina, Taba, Dechencholing, and Chamgang. For sample collection, 250 ml high-density polyethylene (HDPE) containers, properly cleaned (with double distilled water, detergent and 5% nitric acid) and equipped with leakproof caps, were employed following sampling protocol recommended for heavy metal analysis in Standard Methods for the Examination of Water and Wastewater<sup>15</sup>. Each container was appropriately labeled, and to preserve sample integrity, the collected samples were promptly acidified in the field using 5% Nitric Acid, ensuring a pH level below 2.

Subsequently, the acidified samples were transported to the National Water Reference Laboratory, Royal Centre for Disease Control. Upon arrival, the samples were refrigerated and the analyses being conducted the following day to ensure the accuracy and timeliness of results.

#### Water Sample Testing

The Agilent ICP-OES 5110 was used to analyze the samples. A 1000 ppm of multielement calibration standard (Certipur® ICP multi-element standard solution IV) was serially diluted to prepare standard calibration solution as 0.1ppm, 0.5ppm and 1.0ppm in 5% nitric acid matrix. A calibration graph was prepared and the samples were tested sequentially in triplicates.

### Water Pollution Indices

#### **Heavy Metal Pollution Index**

In this study, we employed the Heavy Metal Pollution Index (HPI) as proposed by Mohan et al<sup>12</sup> to assess water quality by considering the presence and significance of heavy metals in water samples. This index has been widely utilized in previous researches to measure the severity of heavy metal pollution in drinking waters<sup>13,16,17</sup>.

The HPI is a tool which consolidates all the heavy metal test readings of the drinking water into a single derived numerical value that reflects the impact of the presence of relevant heavy metals on water quality<sup>12</sup>.

$$HPI = \frac{\sum_{i=1}^{n} W_i Q_i}{\sum_{i=1}^{n} W_i}$$

In this context, Qi represents the subindex of the i-parameter, Wi denotes the weight assigned to the i-parameter, and n signifies the total number of parameters included in the test. The value of Wi for each parameter is inversely proportional to the recommended standard for that specific parameter. The calculation of the subindex for the ith parameter is determined as follows:

$$Q_{i} = \sum_{i=1}^{n} \frac{[M_{i}(-)I_{i}]}{(S_{i} - I_{i})} * 100$$

Mi, Si, and li represent the monitored, standard, and ideal values of the i-parameter for the investigated heavy metals, respectively.

## **Heavy Metal Evaluation Index**

The Heavy Metal Evaluation Index (HEI) is another pollution index associated with heavy metals. It is typically utilized to provide a comprehensive assessment of potential water contamination resulting from heavy metal presence. HEI is computed using the following equation<sup>13</sup>.

$$HEI = \sum_{i=1}^{n} \frac{H_c}{H_{mac}}$$

Here, Hc represents the observed concentration of each i-parameter, while Hmac signifies the maximum permissible level concentration.

## **Methods Assessment**

Before starting the study, it was crucial to assess the methodology's performance, which involved evaluating criteria such as quality control with samples of different concentrations. The accuracy and reproducibility of element measurements via ICP-OES were ensured by analyzing samples in triplicate and constructing calibration curves using a 1000 ppm standard reference material.

## RESULTS

The levels of heavy metals in Thimphu's five primary drinking water sources are detailed in Table 1. This study examined ten

heavy metals, including Aluminum (Al), Boron (B), Barium (Ba), Cadmium (Cd), Chromium (Cr), Copper (Cu), Manganese (Mn), Nickel (Ni), Lead (Pb), and Zinc (Zn). From the results obtained, B was not detected in any of the samples, Cu was not detected in Motithang and Taba, Ni was not detected in Jungshina and Taba, Pb was not detected in Taba and Zn was not detected in Taba and Dechenchholing.

The descriptive statistics, the maximum permissible limit (MPL), limit of detection (LOD) and the wavelengths used for investigating the heavy metal parameters, are summarized in Table 2. The heavy metal concentration of the drinking water in Thimphu are all below the recommended MPL set by the WHO and BDWQS<sup>5,7</sup>.

The Correlation Matrix (CM) analysis was performed to figure out the relationship of the heavy metals detected in the water samples. A CM value closer to 1 indicates a perfect linear relationship between the heavy metals. Table 3 illustrates a correlation between the heavy metals in the water samples. A strong positive correlation of Al (>0.7) is observed with Ba, Mn and Zn. Simiarly, Ba also exhibits a strong positive correlation with Al, Mn, Pb and Zn (>0.7).

The water pollution indices HPI and HEI in this study is calculated using the WHO guideline values for drinking water quality. The mean values of the heavy metals were used to calculate the water pollution indices. Table 4 illustrates the

Table 1. Heavy metal concentration in the major drinking water sources in Thimphu

Source Name		Concentration (mg/l)									
Source maine	Al	В	Ba	Cd	Cr	Cu	Mn	Ni	Pb	Zn	
Motithang	0.0054	nd*	0.0029	0.0004	0.0004	nd*	0.0004	0.0009	0.0043	0.0037	
Jungshina	0.0519	nd*	0.0025	0.0002	0.0003	0.0010	0.0004	$nd^*$	0.0019	0.0007	
Taba	0.0223	$nd^*$	0.0013	0.0003	0.0003	nd*	0.0003	nd*	nd*	nd*	
Dechenchholing	0.0130	$nd^*$	0.0013	0.0003	0.0004	0.0014	0.0001	0.0008	0.0018	nd*	
Chamgang	0.0956	nd*	0.0040	0.0003	0.0003	0.0007	0.0007	0.0010	0.0045	0.0113	

\*Not detected

 Table 2. Descriptive findings of individual heavy metals

Parameter	Number	Mean	Median	Minimum	Maximum	Std. Dev.*	$\mathbf{MPL}^{\dagger}$	Wavelength	LOD <sup>‡</sup>
		mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	λ	
Al	5	0.0376	0.0223	0.0054	0.0956	0.0369	0.9	369.152	0.0051
В	5	0.0000	0.0000	0.0000	0.0000	0.0000	2.4	294.772	0.0008
Ba	5	0.0024	0.0025	0.0013	0.0040	0.0012	1.3	455.403	0.0006
Cd	5	0.0003	0.0003	0.0002	0.0004	0.0001	0.003	214.439	0.0002
Cr	5	0.0003	0.0003	0.0003	0.0004	0.0001	0.05	267.716	0.0003
Cu	5	0.0006	0.0007	0.0000	0.0014	0.0006	2	327.395	0.0005
Mn	5	0.0004	0.0004	0.0001	0.0007	0.0002	0.4	257.610	0.0001
Ni	5	0.0005	0.0008	0.0000	0.0010	0.0005	0.07	231.604	0.0022
Pb	5	0.0025	0.0019	0.0000	0.0045	0.0019	0.01	220.353	0.0021
Zn	5	0.0031	0.0007	0	0.0113	0.0048	5	213.857	0.0022

\*Standard deviation; †Maximum permissible limits; ‡Limit of detection

Table 5. Correlation between nearly incluis in analyzed water samples										
	Al	В	Ba	Cd	Cr	Cu	Mn	Ni	Pb	Zn
Al	1.000									
В	0.000	1.000								
Ba	0.730	0.000	1.000							
Cd	-0.565	0.000	0.038	1.000						
Cr	-0.818	0.000	-0.539	0.508	1.000					
Cu	0.192	0.000	-0.155	-0.756	0.100	1.000				
Mn	0.803	0.000	0.942	-0.050	-0.765	-0.290	1.000			
Ni	0.126	0.000	0.491	0.357	0.398	0.100	0.204	1.000		
Pb	0.376	0.000	0.859	0.305	-0.039	-0.060	0.645	0.811	1.000	
Zn	0.766	0.000	0.905	0.037	-0.426	-0.131	0.835	0.644	0.792	1.000

Table 3. Correlation between heavy metals in analyzed water samples

HPI and HEI values of the water sources. The HPI value of 100 is considered as a critical pollution concentration with respect to heavy metals. HPI results were classified as low (<15), medium (15–30), or high (>30) pollution. As shown in Table 4, all water sources had HPI value of <15, the sources are classified as low pollution by heavy metals. The lowest HPI calculated is the water sample from Taba, indicating a very low heavy metal pollution.

Table 4. Values of pollution indices

P								
Source Name	HPI*	HEI <sup>†</sup>						
Motithang	8.9715	0.5935						
Jungshina	4.3188	0.3230						
Taba	3.5367	0.1324						
Dechenchholing	4.8031	0.2991						
Chamgang	7.4876	0.6661						

\**Heavy metal pollution index;* <sup>†</sup>*Heavy metal evaluation index* 

The classification of overall drinking water quality as per HEI is low (<1.24), medium (1.24–2.48) and high (>2.48) polluted (11). As shown in Table 4, HEI values of all sources are low (<1.24) indicating low pollution by heavy metals.

# DISCUSSION

The study analyzed ten heavy metals in Thimphu's drinking water sources and found all concentrations to be below MPL set by the BDWQS and WHO, indicating compliance with national and international drinking water quality standards<sup>5,7</sup>. Similar studies conducted in Nepal, Bangladesh and Pakistan all exceeded the MPL<sup>18-20</sup>. The contamination with heavy metals may be attributed to poor waste management, open sludge discharge, lack of municipality measures, electronic waste, pesticide application and mining activities<sup>21,22</sup>. These anthropogenic activities are very small to none in Thimphu. The Correlation Matrix analysis revealed significant correlations between heavy metals in the water samples, suggesting common sources or geochemical associations<sup>23</sup>. For instance, Aluminum showed strong positive correlations with Barium, Manganese, and Zinc, while Barium exhibited strong positive correlations with Aluminum, Manganese, Lead, and Zinc. These findings have implications for future investigations.

The study computed HPI and HEI indices to evaluate the overall quality of drinking water sources in Thimphu. All sources showed low pollution levels with HPI and HEI values classified as low, while similar studies in India revealed higher HPI and HEI values, indicating potential contamination in their water sources<sup>24,25</sup>.

These findings offer reassurance, indicating that the drinking water from the primary sources in Thimphu complies with the WHO guidelines for heavy metal pollution. However, more in-depth analysis with statistical methods like Ward's hierarchical cluster analysis as well as principal component analysis should be conducted to elucidate the strengths and weakness of the findings in future<sup>26</sup>.

# LIMITATIONS

One of the primary challenges in assessing heavy metal concentrations in water sources is the dynamic nature of environmental factors. Seasonal changes, characterized by alternating dry and wet periods, play a pivotal role in altering the composition of water bodies<sup>27</sup>. During the wet season, increased precipitation and runoff can dilute the concentration of heavy metals in water sources, potentially leading to underestimations of pollution levels. Conversely, in the dry season, reduced water flow and increased evaporation can concentrate heavy metals, resulting in potentially misleading higher readings<sup>28-30</sup>. Streams with low discharge are particularly susceptible to the influence of seasonal variations. These water bodies are more vulnerable to the accumulation of heavy metals due to reduced dilution capacity during dry periods<sup>31</sup>. Consequently, in this study a

sample collection during a low-flow period in the month of March, 2023 may not accurately represent the typical heavy metal concentrations in the water source.

# CONCLUSIONS

In conclusion, the study demonstrates that the levels of heavy metals in Thimphu's primary drinking water sources are generally within acceptable limits, as per WHO standards. The low pollution indices (HPI and HEI) further support the conclusion that these sources have low heavy metal pollution. However, the positive correlations observed between certain heavy metals should prompt further investigation to identify potential sources and address any long-term concerns related to water quality. Considering the limitations mentioned, a singlesample approach, while valuable for initial assessments, may not provide a comprehensive understanding of the long-term quality and potential risks associated with the water sources. Therefore, regular monitoring and continued research in this area are essential to ensure the safety and sustainability of Thimphu's drinking water supply.

# REFERENCES

- World Health Organization. Drinking-water [internet]. 2023 [cited 2023 Sep 27]. [Full Text]
- Steenland K, Barry V, Anttila A, Sallmen M, Mueller W, Ritchie P, et al. Cancer incidence among workers with blood lead measurements in two countries. Occup Environ Med. 2019;76(9):603-10. [PubMed | Full Text | DOI]
- Houston MC. The role of mercury and cadmium heavy metals in vascular disease, hypertension, coronary heart disease, and myocardial infarction. Altern Ther Health Med. 2007;13(2):S128-33. [PubMed | Full Text]
- Rzymski P, Tomczyk K, Rzymski P, Poniedziałek B, Opala T, Wilczak M. Impact of heavy metals on the female reproductive system. Ann Agric Environ Med. 2015;22(2):259-64. [PubMed | Full Text | DOI]
- 5. World Health Organization. Guidelines for drinking-water quality: fourth edition incorporating the first addendum. Geneva: World Health Organization 2017. [Full Text]
- USEPA (U.S. Environmental Protection Agency). Ground Water and Drinking Water: National Primary Drinking Water Regulations [internet]. 2023 [cited 2023 Oct 26]. [Full Text]
- National Environment Commission. Bhutan Drinking Water Quality Standard. Thimphu, Bhutan: National Environment Commission. 2016. [Full Text]
- Fernández-Luqueño F, López-Valdez F, Gamero P, Luna S, Aguilera-González EN, Martinez A, et al. Heavy metal pollution in drinking water-a global risk for human health: A review. 2013;7:567-84. [Full Text]

- Kapoor D, Singh MP. Heavy Metals in the Environment. Elsevier; 2021. Heavy metal contamination in water and its possible sources. p. 179-89. [Full Text | DOI]
- National Statistics Bureau of Bhutan. 2017 Population and housing census of Bhutan. Thimphu: Royal government of Bhutan; 2018. [Full Text]
- National Statistics Bureau of Bhutan. Population projections for Bhutan 2017-2047. Thimphu: Royal Government of Bhutan; 2019. [Full Text]
- Mohan SV, Nithila P, Reddy SJ. Estimation of heavy metals in drinking water and development of heavy metal pollution index. J of Environ Sci Health Part A: Environmental Science and Engineering and Toxicology. 1996;31(2):283-9. [Full Text | DOI]
- Herojeet R, Rishi MS, Kishore N. Integrated approach of heavy metal pollution indices and complexity quantification using chemometric models in the Sirsa Basin, Nalagarh valley, Himachal Pradesh, India. Chin J Geochem. 2015;34(4):620-33. [Full Text | DOI]
- Rezaei A, Hassani H, Hayati M, Jabbari N, Barzegar R. Risk assessment and ranking of heavy metals concentration in Iran's Rayen groundwater basin using linear assignment method. Stochastic Environmental Research and Risk Assessment. 2018;32. [Full Text | DOI]
- Baird R, Rice E, Eaton A. Standard methods for the examination of water and wastewaters 2017. 23rd ed. American Public Health Association, Water Environment Federation, Washington DC. 2017. [Full Text]
- Afonne OJ, Chukwuka JU, Ifediba EC. Evaluation of drinking water quality using heavy metal pollution indexing models in an agrarian, non-industrialised area of South-East Nigeria. Journal of Environmental Science and Health, Part A. 2020;55(12):1406-14. [PubMed | Full Text | DOI]
- Gupta A, Singh R, Singh P, Dobhal R. Heavy Metals in Drinking Water Sources of Dehradun, Using Water Quality Indices. Analytical Chemistry Letters. 2017;7(4):509-19.
   [Full Text | DOI]
- Warner N, Levy J, Harpp K, Farruggia F. Drinking water quality in Nepal's Kathmandu Valley: A survey and assessment of selected controlling site characteristics. Hydrogeology J. 2008;16:321-34. [Full Text | DOI]
- Rahman M, Rima SA, Saha SK, Saima J, Hossain MS, Tanni TN, et al. Pollution evaluation and health risk assessment of heavy metals in the surface water of a remote island Nijhum Dweep, northern Bay of Bengal. Environmental Nanotechnology, Monitoring & Management. 2022;18:100706. [Full Text | DOI]
- Hayder R, Hafeez M, Ahmad P, Memon N, Khandaker MU, Elqahtani ZM, et al. Heavy Metal Estimation and Quality Assurance Parameters for Water Resources in the Northern Region of Pakistan. Water. 2023;15(1):77. [Full Text | DOI]

- Rehman K, Fatima F, Waheed I, Akash MSH. Prevalence of exposure of heavy metals and their impact on health consequences. J Cell Biochem. 2018;119(1):157-84.
   [PubMed | Full Text | DOI]
- 22. Singh Sankhla M, Kumari M, Nandan M, Kumar R, Agrawal P. Heavy Metals Contamination in Water and their Hazardous Effect on Human Health-A Review. Int J Curr Microbiol App Sci. 2016;5:759-66. [Full Text | DOI]
- Santosh Kumar S, Paulo JCF, Dibyendu R, Satpathy KK. Environmental RIsk Assessment of SOil COntamination. 1st ed. Croatia. InTech; 2014. Chapter 25, Geochemical Speciation and Risk Assessment of Heavy Metals in Soils and Sediments. [Full Text | DOI]
- 24. Kumar V, Sharma A, Kumar R, Bhardwaj R, Kumar Thukral A, Rodrigo-Comino J. Assessment of heavy-metal pollution in three different Indian water bodies by combination of multivariate analysis and water pollution indices. Human and Ecological Risk Assessment. 2020;26(1):1-16. [Full Text | DOI]
- Dogra S, Sharma K, Singh N. Water quality and health risk assessment of heavy metals in groundwater of Ranbir Singh Pura tehsil of Jammu and Kashmir, India. Environ Monit Assess. 2023;195(9):1026. [PubMed | DOI]
- Kowalska JB, Mazurek R, Gąsiorek M, Zaleski T. Pollution indices as useful tools for the comprehensive evaluation of the degree of soil contamination-A review. Environ Geochem Health. 2018;40(6):2395-420. [PubMed | Full Text | DOI]

- 27. Ali M, Bhuyan M, Arai T. Seasonal variation of trace elements in water and sediment of the Turag and Balu Rivers, Bangladesh. Egyptian J Aquatic Biol Fisheries. 2022;26:513-40. [Full Text | DOI]
- Eliku T, Leta S. Spatial and seasonal variation in physicochemical parameters and heavy metals in Awash River, Ethiopia. Appl Water Sci. 2018;8(6):177. [Full Text DOI]
- 29. Sharma R, Singh NS, Singh DK. Impact of heavy metal contamination and seasonal variations on enzyme's activity of Yamuna river soil in Delhi and NCR. Appl Water Sci 2020;10(3):83. [Full Text | DOI]
- Guo W, Zou J, Liu S, Chen X, Kong X, Zhang H, et al. Seasonal and Spatial Variation in Dissolved Heavy Metals in Liaodong Bay, China. Int J Environ Res Public Health. 2022;19(1). [PubMed | Full Text | DOI]
- 31. Edokpayi JN, Odiyo JO, Popoola EO, Msagati TAM. Evaluation of temporary seasonal variation of heavy metals and their potential ecological risk in Nzhelele River, South Africa. Open Chemistry. 2017;15(1):272-82. [Full Text | DOI]

#### AUTHORS CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

CD: Concept, design, data analysis, manuscript editing and writing

PC: Data collection and analysis, manuscript editing and review

Author agree to be accountable for all respects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

## CONFLICT OF INTEREST

None

GRANT SUPPORT AND FINANCIAL DISCLOSURE

None