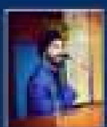
"COMPARATIVE STUDY ON BIOMONITORING OF HEAVY METALS POLLUTION IN SIRPUR LAKE AND BILAWALI TANK USING BIVALVES AS BIOINDICATOR"

About Authors

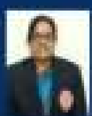
Dr. Mohd. Abbas Mantoo

In the intricate tapestry of scientific exploration, Dr. Abass Mantoo emerges as a beacon of brilliance and achievement. His contributions to the fields of molecular toxicology and zoology are nothing short of remarkable, reflecting a life devoted to the pursuit of knowledge and discovery. Dr. Mantoo's journey to this pinnacle of success is a testament to his unwavering dedication, an unquenchable passion for understanding the intricacies of the natural world, and a relentless pursuit of excellence. It's a journey that has not only shaped the course of his own life but has also left an indelible mark on the scientific community. Throughout his illustrious career, Dr. Mantoo has garnered a constellation of accolades and recognitions that highlight the depth of his capabilities. Notably, he was bestowed with the prestigious Young Scientist Medal by the Zoological Society of India, a highly coveted honor that acknowledges his pioneering work in the realm of zoology. This award is more than a recognition, it signifies his profound impact on the field and his contribution to advancing our understanding of the animal kingdom. Furthermore, in the field of Molecular Toxicology, Dr. Mantoo was honored with the esteemed Gold Medal, a symbol of his extraordinary expertise and groundbreaking research in this critical scientific discipline. It is a recognition of his contributions to our understanding of the complex interactions between biological systems and harmful agents, a vital area of study with far-reaching implications.



Prof. Shailendra Sharma, *MSc., PhD., DSc. Principal, Adarsh Institute of Management and Science, Dhamnod.*

Dr Sharma has 27 years of academic experience and 35 years of research experience. 98 of your research papers have been published in national and international journals. You have written 5 books and you are a member of many academic institutions as well as a member of the Working Council of the Zoological Council of India. You have been awarded by the Zoological Council of India for Lifetime Achievement. Similarly, you have been awarded by many national and international institutions for your amazing work in research. Under your guidance, 15 students have earned PhD degrees and as well as researchers have earned MPhil degrees. You have completed the research management of three UGCs research projects. You have also been awarded the fellowship of the European Commission. You have worked as a water scientist in IIT Roorkee, as well as a water policy expert and you are also known as an expert in nutrition and yoga.



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She has an experience of more 40 Years in Teaching and Research. She has Published two Article in books, more than 15 Research Paper Published in International Journals, 20 Abstracts in proceedings and guided 8 Ph.D. and 10 M.Phil. students. Appointed as chairman of Board of studies in D.A.V.V. Indore & member of board of studies in B.R. Ambedkar University of Social Sciences. Now working as counsellor at Indira Gandhi National Open University (IGNOU). She has received Zoological Congress Medal 2023 awarded by Zoological Society of India.

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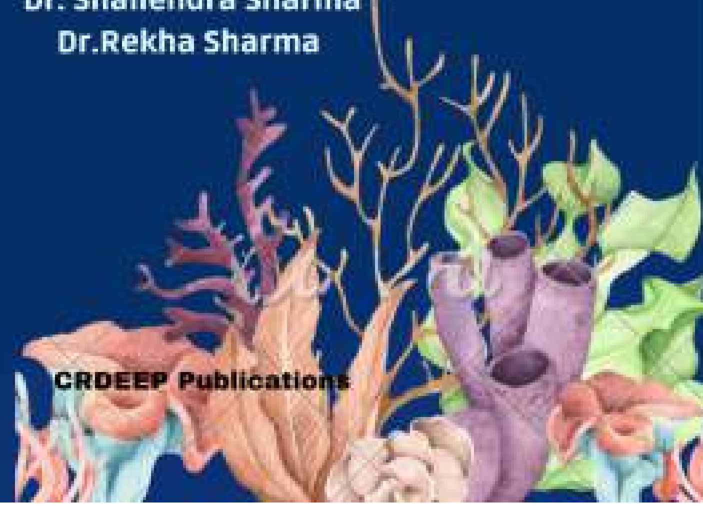

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"COMPARATIVE STUDY ON BIOMONITORING OF HEAVY METALS POLLUTION IN SIRPUR LAKE AND BILAWALI TANK USING BIVALVES AS BIOINDICATOR"

THESIS

Submitted for Doctor of Philosophy in Zoology
(Faculty of Life Science)
Zoology

To
DEVI AHILYA VISHWAVIDYALAYA, INDORE (M.P.)

2019

Submitted by
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CERTIFICATE OF THE SUPERVISOR

This is to certify that the work entitled **“COMPARATIVE STUDY ON BIOMONITORING OF HEAVY METALS POLLUTION IN SIRPUR LAKE AND BILAWALI TANK USING BIVALVES AS BIOINDICATOR”** is a piece of research work done by **Shri Mohd Abass Mantoo** under our guidance and supervision for the degree of Doctor of Philosophy of Devi AhilyaVishwavidyalay, Indore, (M. P.) India that the candidate has put –in an attendance of more than 200 days with me.

To the best of my knowledge and belief the Thesis

1. embodies the work of the candidate himself,
2. has duly been completed,
3. fulfills the requirements of the ordinance relating to the Ph.D. degree of the University, and
4. is up to the standard both in the respect of contents and language for being referred to the examiner.

Signature of Co-supervisor **Signature of Supervisor**

Signature of Principal

DECLARATION BY THE CANDIDATE

I declare that the thesis entitled **"COMPARATIVE STUDY ON BIOMONITORING OF HEAVY METALS POLLUTION IN SIRPUR LAKE AND BILAWALI TANK USING BIVALVES AS BIOINDICATOR"**is my own work conducted under the supervision of **Dr. Shailendra Sharma** Principal, Adarsh Institute of Management and Science (Supervisor) and **Dr. Rekha Sharma** Professor, Department of Zoology (Co-supervisor) at Govt Holkar Science College Indore Madhya Pradesh approved by Research Degree Committee. I have put in more than 200 days of attendance with the supervisor at the Centre.

I further declare that to the best of my knowledge the thesis does not contain any part of any work which has been submitted for the award of any degree either in this university or in any other university/Deemed university without proper citation.

Signature of the Co-Supervisor **Signature of the Supervisor**

Signature of the candidate

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&Incharge, Central pollution control board, Ministry of environment & Forest (Government of India). This research work could not be completed without their help.

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Mohd Abass Mantoo

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INTRODUCTION

INTRODUCTION

Human population is increasing rapidly in the world. To fulfill the requirement of their needs, anthropological activities are progressively increasing. Water is an elixir of life, so urbanization near water bodies are establishing. Settlement of population needs leads to technology development, industrialization, farming, and many more activities. These all have been adversely affecting environment and ecosystem. Quality of water changes due to pollution during human settlements. Waste water effluent comprising heavy metals, which leads to degradation of water quality and also adversely affect to ecosystem of such water bodies. Heavy metals viz. As, Zn, Cu, Pb Cd, Hg, Ni and Cr are some trace metals present in polluted water. Higher concentrations of these heavy metals make harmfully affect to population of water bodies. Heavy metals are non-biodegradable and are persistent in nature. It transfers through accumulation in lower trophic organisms to higher trophic organism through food chain and leads to bad effect on ecological balance of environment and diversity of aquatic organisms. Heavy metals can accumulate by algae as primary consumer and transmitted across the higher levels of the food chain results into spreading of pollutants from origin to human. Occurrence of toxic metals in lakes, ponds, dams, ditches and river water affect the lives of people that depend upon these water sources for their daily requirements. Heavy metals like arsenic, cadmium, mercury, lead, copper, zinc may cause a variety of adverse health effects to humans. Considering the use of water reservoirs as water supplies, threaten are thus posed on human health through drinking water, polluted vegetable and foodstuff etc. Study of bioaccumulation as biomarkers is a sensitive and conventional method. Biomonitoring is a systematic use of biotic organisms to determine the changes in water quality, by analyzing bioaccumulation, biological effects over health and physiology. The use of bioaccumulation surveys to determine pollutant levels is common in marine and freshwater ecosystems, since the calculation of the pollutant conce ntration in the biotic organism is the only way to assess the bioavailability of a pollutant in the sorrounding environment.

Mollusks organisms have been studied in order to identify their role as biomoni toring organisms. There are different attributes, which make mussels suitable organisms for environmental monitoring including wide geographical distributing, abundant, sedentary, tolerant to environmental contaminants, high bioconcentration factors of pollutants, very low-level metabolizing enzyme activities of organic contaminants, reasonable size, reasonably long-lived, Strong enough to live in laboratory and field experiments etc. Physicochemical parameters include dissolved oxygen, temperature, Ph, hardness, salinity and organic component. An organism's feeding behavior, respiration, mobility, and were and how it lives are key biological factors affecting metals uptake and the toxicity of metals contaminated sediments. Metal concentrations are represented as concentration per unit body weight (µg / g), then the highest value can be assumed to be reported among the smallest individuals and thus could be misleadingly interpreted. The body burdens of metals in bivalves have been used to identify and locate areas with exceedingly more levels of trace metals and organic pollutants hence they can be used as bio-monitors for aquatic environment. Biowater Accumulation Factor (BWAF) and Bio-sediment Accumulation Factor (BSAF) can be used to estimate the ability of bivalve to absorb metals from water / sediment into the tissues of body. Through comparing the BWAF / BSAF values, one can examine the ability of various bivalve organisms to absorb metals in their body tissues from water and sediments. Bioaccumulation only detects the levels of heavy metals, in aquatic organisms, but a true evaluation of damage inflicted by heavy metals should come from comprehensive biomarker studies. Biomarkers are more significant than bioaccumulation because they deal at molecular, cellular, tissue/organ, physiological changes on the organism level and assess contamination based on a direct measure of change in the organism. Modulation of the enzymatic antioxidant enzyme function also involves the development of catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST), reduced glutathione (GSH), malondialdehyde (MDA) and glutathione peroxidase (GPx). While extensive work on biomarkers research is being undertaken in several parts of the world, such studies are yet to receive sufficient attention in India. Freshwater bivalve species such as, *Unio* sp., *Lamellidens corrianus, Lamellidens marginalis* which represent the inwater bodies will be the best organisms for this study. Important biochemical constituents such as protein, ascorbic acid and DNA were also used as susceptible biomarkers for the biomonitoring of heavy metal pollution in freshwater ecosystem. Sirpur Lake and Bilawali Tank is under constant pressure from anthropogenic contaminants that derive from different sources in the catchment areas.. Its water has great utility for various purposes in nearby areas. So it is urgency to access the accumulation of heavy metals in the water of both reservoirs. A literature survey disclosed that most investigators studied the reservoir's physico-chemical parameters and never performed a biomonitoring test to detect the extent of heavy metal contamination in water, sediment and inhabiting biota, so the present study was conducted. The knowledge of concentration of metal in native species is very important with respect to nature management, human consumption of these species and to find out the most useful biomonitor species. Therefore, in the present study different native species of freshwater bivalves, *Unio* sp., *Lamellidens corrianus*, *Lamellidens marginalis* all were selected to establish a local environmental monitoring network using bivalves as bioindicator species to evaluate the trends of bioaccumulation of Zn, Cu, Pb Mn Hg and Cd in freshwater ecosystem. Following objectives were undertaken in this study:

1. To determine the level of heavy metals Zn, Mn Hg Cu, Cd and Pb in surface water, soil sediments and whole soft body tissues of three bivalve species viz. *Unio sp*., *Lamellidens corrianus*, *Lamellidens marginalis* inhabiting in two reservoirs of Indore district during different seasons.
2. To find out most appropriate bivalve species as sentinel animal for monitoring of metal pollution in the freshwater ecosystem.

3. To measure the impact of heavy metals on biomarker oxidative stress indicator parameters like activity of antioxidant enzymes, glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase(CAT), and Glutathione-S-transferase and level of reduced glutathione (GSH) of three bivalve species *Unio sp*., *Lamellidens corrianus*, *Lamellidens marginalis* inhabiting the listed reservoirs of the district of Indore during various seasons.
4. To measure the impact on biochemical composition (protein, ascorbic acid RNA and DNA content) of heavy metals in soft body tissues of three bivalve *Unio* sp., *Lamellidens corrianus*, *Lamellidens marginalis* inhabiting the two reservoirs of Indore district during different seasons.

REVIEW
OF
LITERATURE

REVIEW OF LITERATURE

Macro-invertebrates are furthermost demanding in bio-monitoring studies giving well responses to organic and inorganic pollution as reported in earlier studies have been extensively documented (Thorne and Williams, 1997; Kazanci and Dugal, 2000). Micro-invertebrates have sensitive life stages, which respond to stress and integrate effects of short-term as well as long-term environmental stressors. This is due reason of importance of micro-invertebrates in maintaining biodiversity (Meyer *et al.*, 2007; Richardson and Danehy, 2007). Macro-invertebrates studies also provide a developed method to determine water quality of River, Lakes and other water bodies on collection and identification of benthic macro-invertebrates. Varshney *et al.*, 1976; Rao *et al.*, 1985; Sunny and Diwan, 1991; Sharma, 2003; Sharma *et al*., 2007 previously studied to find the diversity of benthic macro-invertebrates in Malwa region of Madhya Pradesh.

Indore is a largest city of Madhya Pradesh in central India. Holkar Empire ruled this. This is situated on latitude 22020" N to 23005" N and longitude 75025" E to 75015" E. There are many water bodies in this city and fulfill the water requirements of the population of this city. Bilawali tank is one of them and situated in the south-west direction of Indore at Khandwa road near Asaram Bapu Ashram followed by a distance of 6 km from Indore, Madhya Pradesh. This Lake is spread in 69ha with 117 ha catchment area. This is a man made earthen tank developed by Maharaja Tukoji Rao Holkar under the supervision of Sri Geddes in year 1914. This tank was then connected with Pipliya Pala Tank through a canal near the Limbodi village to fulfill the drinking, fishing, culture etc. of a contemporary residence of Shri Bhojket.

Sirpur Lake is situated in southwest area of Indore and is surrounded by large trees on half its bank and the rest, by shrubs and agriculture land. Domestic sewage and some small-scale industries sewage are merged in this lake. Human settlement near this lake uses this water for agriculture and some how domestic purposes. This lake is a big collector of rainwater. A dense population of benthic communities and shorebirds are makes this Lake beautiful. Tourists are vising this lake to see different birds and natural track. Bank area of this tropical lake is an ideal habitat for birds as well as benthic community. This plays an important role in exchange of allochthonous and autochthonous food cycles in the lake ecosystem.

Presence of heavy metals in ponds, lakes, dams, ditches, rivers and other water bodies affects the population depend on these water bodies for their needs. Consumption of aquatic food of those water bodies also toxic and are hazards to health. These can also be transferred through food chain (Choudhary *et al.,* 2014). A quality potable water supply is also a challenge in this century to fulfill the all requirement population (Schwarzenbach *et al.,* 2010). Different study has shown that metals have ability to bioconcentrate in organisms directly from the water and bioaccumulate within food chains. This may causes the higher trophic organisms to suit contaminated with higher concentrations of toxic metals (Hargrave *et al.*, 2000; Lee *et al.*, 2000; Boran & Altinok 2010; Shariati *et al.*, 2011). This is due heavy metal pollution poses a great potential threat to the human health, ecosystem and environment. There is an urgent need of monitoring of them, not only to prevent diseases and hazards, but also to check the water bodies from going further polluted. Heavy metal contamination is being recognized as melodramatic in large parts of the developing countries, particularly in India, China and others (Meharg, 2004; Cheng, 2003). Contamination done by non-essential chemicals or/and elements in dietary substances is a series and adverse effects on human body and animals (D'Souzz and Peretiatko, 2004). Heavy metals are ubiquitous and recalcitrant; their entry into human body poses a probable health risk. Metals can disturb the control mechanisms viz. homeostasis, transport of blood, compartmentalization and binding to specified cell constituents. Heavy metals can cause flopping of the cellular processes as displacement of essential metals from their respective locations. Oxidative deterioration of biological macromolecules has been found first due to binding of metals to DNA, RNA and nuclear proteins (Flora *et al.*, 2008). There are many symptoms are often the indicators of contamination and they are helps to identify the real contaminant. Intellectual disability in children, central nervous system disorders, dementia in adults, kidney diseases, liver diseases, emotional instability, insomnia, depression and vision disturbances are some symptoms that arise as a result of metal poisoning in human (Flora *et al.*, 2008; Jan *et al.*, 2011). Toxicity accompanying through exposure to metals, if unrecognized or inappropriately treated epitomizes a clinically significant medical problem and having greater impact on increasing morbidity and mortality rate in affected population. Due to huge applicability of toxic metals in domestic, industrial and agricultural purposes gives adverse effects in the environment and raises serious anxieties over their potential health effects on humans. Toxicity that arises by a sudden exposure to substantial quantities of metals affects multiple organ systems. Severity of health consequences of toxic metals depends on type and form of element, route and duration of exposure and to a greater extent, on individual susceptibility (Jan *et al.*, 2011). Metals are primarily different oxidative stress in human body viz. increased Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS) production; depletion of intracellular antioxidant stores, free-radical scavengers; inhibition of activity of enzymes that contribute significantly to the metabolism and detoxification of reactive oxygen species.

World health organization estimate about eighty percent of potable water of India is polluted due to domestic waste. This improper management of water structures may cause serious difficulties in availability of drinking water (Subha, 1995). Water bodies are also most polluted through industrial effluents. Discharges of wastewater of different industry are discharged with out treatment in to water bodies. These can alter the physical, chemical and biological characteristics of water in such a way that they are not useful for the purpose for which they are planned (Noorjahan *et al.,* 2002). Water quality is an important part in wetland habitat evaluation. This is a host of interacting physical and chemical factors and can influence the levels of the primary productivity and influence the trophic structure and total biomass throughout the aquatic food network (Wetzel, 1983). Trace metals are a large group of metallic elements. These are available in limited amounts in nature and as well as in living organisms. These are among the major concerns in wastewater pollution (Salchi *et al.,* 2008). Heavy metals are comes out from heavy industry viz. electroplating, battery factories, mining operations, insulation, rubber, plastic and others. Chromium and cadmium are especially dangerous to aquatic organisms and can be bio-accumulated in food chain are most discharged from industries effluents (Medina *et al*., 1986; Brown and Louma, 1995; Pip, 1995). These are metallic contaminants effects on essential function in human physiology (Barak and Mason, 1990). Fishes and bivalves are accumulating metals in their bodies and constitute an human food (Lopez-Artiguez *et al.,* 1989; El-Deek *et al.,* 1994; Schuhmacher and Domingo, 1996; Zyadah, 1996; Sidoumou *et al.*, 1997). These are conceivably an indirect source of metals entering the human body. They are not only transfer those metals but may also suffer from a wide range of metabolic, physiological and ecological issues. Concentration of metal increases, influences the accumulation of metal and its damage effect (Cain and Louma, 1986; Buschiazzoa *et al.*, 2004). Long term of metal exposure gives cumulative effects of metals and chronic poisoning. Different reports on the occurrence and accumulation of trace metals in bivalve have lead to concern the contamination of commercial mussel (Pentreath, 1973; Valiela *et al.*, 1974). Hemelraad *et al.* (1986; 1987; 1988) have published a series of research articles connected to effect of cadmium on freshwater clams and reported their adverse effects on their health. Hemelraad *et al.* (1990) studied on effects of cadmium on freshwater clams *Anodonta cygnea* and interaction of Cd with the essential elements Na+ (sodium), K+ (potassium), Ca_2+ (calcium), Hg2+ (Mercury), Fe2+ (Ferrus) and Zinc reported potential decreases of health and increased bioaccumulation. Abdel Moati and Farag (1991); El-Fayomy (1994) studied the rate of bioaccumulation in Edku Lake and found the marine clam *Cardium edula* in Lake accumulated more heavy metals than examined controlled fishes.

Aquatic ecosystems always excel the pressure of anthropogenic pollutants of numerous sources located at catchment areas or adjoining places. Toxic pollutants present in pollutants are sometimes lethal or sub lethal and they deteriorate the live of such ecosystem. Toxic effects of pollutants are depends on the type of pollutant and on its concentration. Concentrations of the pollutants are low, causing only sub-lethal or chronic disease nevertheless, acute massive pollution resulting fish-kill or death of various organisms, may also occur in rivers or lakes (Farkas *et al.,* 2007; Diagomanolin *et al.,* 2004; Singh *et al.,* 2004). Heavy metals are a distinct group of contaminants introduced into aquatic system from volcanic eruptions, weathering of soil rocks and from anthropogenic activities involving mining, processing and use of metals and/or allied metal contaminants (Karageorgis *et al.,* 2003). Present of heavy metals in environment at natural level had never been a threat to health and ecosystem but increased industrial activities leading to air born emissions, auto exhausts, effluents, solid waste dumping have become the sources of large quantities of heavy metals into the environment which leads to trouble (Mhatre, 1991). When trace metals are entering into natural water, it becomes a part of water-sediment system. Distribution processes of this sediment system are controlled by a self-motivated set of physical-chemical interactions and equilibrium. River sediments are major components of environment, which provides nutrients for living organisms, and serve as bowl for deleterious chemical species and reflect the history of the river pollution (Singh *et al.*, 2003). Sediments are acting as carriers as well as sinks for contaminants in aquatic environments. Heavy metals contaminants are basic environmental pollutants present in sediment of aquatic ecosystem. Occurrence of these pollutants in waters and biota indicate the presence of natural or anthropogenic sources. Earlier reports told that the concentrations of trace metals in suspended and bed sediments are sensitive indicators of contaminants in hydrological systems (Diagomanolin *et al.*, 2004; Jain, 2004; Idris *et al.*, 2007). Particle size, composition and organization of sediment are affected by the presence of heavy metals. Heavy metals may be adsorbed to sediments and/or accumulated in benthic organism. Mobility, bioavailability and subsequent toxicity of metals in aquatic system are now a major research area (Ghabbour *et al.*, 2006). A study was performed in Pearl River Delta, South China for metal and nutrient concentrations in sediments and reported there was a serious contamination of Cd, Pb, and Zn in sediment. This was then further classified by Hong Kong Environmental Protection Department (Cheung *et al.*, 2003). Farkas and associates (2007) reported significantly increased pollution of heavy metal Cd, Cu, Ni, Pb and Zn in surface sediments in near inlet of the River Lambro. Singh and his associates worked on monitoring of heavy metals in Gomati Nagar with 24 different water assessment parameters over 5 years and reported significant pollution in Gomati River. Heavy metals are considered as the most important pollutants in aquatic ecosystem due to their highly bio accumulative nature, persistent behavior and potential of higher toxicity (Niyogi and Wood, 2004; Censi *et al.*, 2006). Biochemical composition of water bodies are varies according to seasonal changes, environmental factors viz. temperature, salinity, starvation and toxicants in water (Verlecar *et al.*, 2007 Nandurkar and Zambare, 2010; Salaskar and Nayak 2011). Changes in biological functions, structures and proteins of species in response to metal pollution may be used to assess the health of aquatic animals and provides the early warnings of different environmental risks (Venier and Zampieron, 2005). Mahajan (2007) studied the changes in biochemical composition of gill, foot, digestive gland and whole soft body tissues of freshwater bivalve, *Lamellidensmarginalis* exposed to chronic concentration of arsenic trioxide, cadmium chloride and lead chloride reported and confirms metal accumulation. Injal and Raut, (2009) worked on lead induced alterations in protein levels of gills and mantle of freshwater bivalve, *Lamellidensmarginalis* and reported acute bioaccumulation. Seasonal variations in RNA content in *Austrovenusstutchburyi* at different sites of water bodies were studied by Norkko and Thrush (2006) and reported altered RNA content. Singh *et al.*, (2010) studied the effect of sublethal treatment of deltamethrin pesticide on protein, amino acid and nucleic acids levels in gonadal, nervous and foot tissue of *Lymnaea acuminate* and reported irregular ratio of such parameters. Under some certain unfavorable conditions, the organism develops positive adaptive methods such as mobilization of energy from reserves to tide over the crisis and to protect themselves (Bryan *et al.*, 1986). Study of biochemical components would be much expressive to estimate the nutritive value of an organism, and its further analysis with the metal effect would gives an complex relation between metal pollution and metabolism of biochemical constituents. Water is the most important part of environment. This is a direct approach to reveal heavy metal pollutant. Bio monitoring is a best scientific approach to assess the environment comprising human exposures to natural and synthetic chemicals, grounded on sampling, analysis and interpretation of an individual organism's tissues and fluids. These results the measurements of natural and manmade chemicals that have entered and remained in the organisms and their corresponding effects induced (Zhoua *et al.*, 2008). Bio-monitoring is an approach to assessment the risk of environmental and occupational exposures, which provides an estimate of total quantity absorbed and gives indirect access to determination of focus site concentrations. This is reveals the presence of pollutants and also measure their toxic effect biological indicators. Active and passive monitoring are basic approaches to assess the pollutants and their toxic effects at different stage from species to community level of any ecosystem (Ron *et al.*, 2003). A plant or an animal, which reveals the presence of substance in its vicinity by showing some typical symptoms, which can be distinguished from the effects of other natural or anthropogenic stresses, is bio indicator (Mhatre, 1991). Bio-indicator is showing the earliest responses to pollutants, which enabling to indicate the presence and predict the consequences of undesirable anthropogenic effects (Salanki, 1986). Movement of trace element through aquatic food web are require to understand in the means by which aquatic organisms accumulate trace metals from their environment. Many aquatic invertebrates make trophic transfer for total trace element accumulation (Alkarkhi *et al.,* 2008). Trace metals have many metal-binding ligands in which as sulfide are for Ag bio-kinetic changes in bivalves. As dissolved Hg uptake is reduced exposure to metals such as Ag, Cd, Cu, and Zn in mollusks and invertebrates. The tissue body burden and the detoxificatory fate of metals in animals are important in affecting metal accumulation than the nature of the exposure routes. Trace metal accumulation is a variable in natural populations of bivalves with different physicochemical environments and histories of exposure (Jain and Sharma 2001). Water current, water flow, renewal of water, pH, hardness, salinity etc. are different factors affects the distribution of heavy metals in molluscs. Some biological factors viz. greater metabolic rate in small organisms may also account for higher concentration of essential elements Cu and Zn (Zhoua *et al.,* 2008). Body size, weight, sex etc. biological factors of molluscs also play a key factor in bioaccumulation of metals. Lower concentrations of cadmium and zinc concentrations were found in males as compared to females in *Perna perna* species whereas high concentration of cadmium was reported in *Pecten maximus* (Boyden, 1974; Romeo *et al.,* 2000). Different species also accumulated different concentration of metals. Uptake of metals and subsequent bioavailability are also highly dependent on geochemical and biological factors. Major differences in bioaccumulation between bivalve species are seen in different species. Age, size, sex, genotype, phenotype, feeding activity and reproductive state of species can be accumulated differently (Zhoua *et al.,* 2008). Organic carbon, water hardness, temperature, pH, dissolved oxygen, sediment grain size and hydrologic are some geochemical factors that influence bioaccumulation in species (Elder and Collins, 1991). Salinity of sediment or water influences the availability of contaminants whereas temperature changes can vary bivalve spawning, which will affect the soft tissue mass and lipid composition of the mollusk (Biliaff *et al.,* 1997). Uptake and accumulation in deposit feeders can be correlate to metal concentrations in sediments, whereas accumulation in filter feeders are most likely reflect metal concentrations in water (Newman and McIntosh, 1982). Metallothioneins may be trapped in whole soft tissues in bivalves. Induction of metallothioneins binding cadmium in various soft tissues, gills, labial pulps, digestive gland and others tissues of bivalve has been studied by numerous researchers (Bebianno *et al.,* 1993; Biliaff *et al.,* 1997). Concentration of high environmental phosphate may facilitate the uptake of cadmium by organisms (Romeo *et al.,* 2000). Antagonism between zinc and cadmium has been reported on many organisms. Concentration of toxic metal in molluscs are not only depends on level of the element in environment but also on factors viz. size, age, speed of growth, sex and reproductive conditions of the molluscs, season, salinity, chemical species and interaction with other pollutants (Biliaff *et al.*, 1997).

The freshwater bivalve *Caelatura (Caelatura) companyoi* and the snail *Cleopatra bulimoides* of River Nile (Assiut of Upper Egypt to Damietta branches of Lower Egypt) were chosen by Salchi *et al.*, 2008 in their studies and determine their ability for the accumulation of Cr_3+ and Cd_2+ in their soft parts and to show the extent of their tolerance towards these pollutants in the freshwater ecosystems. They reported that the possibility of using these organisms as bioindicators for heavy metals pollution and provide basic information for detecting the current status of heavy metal pollution in such freshwater ecosystems. Sharma *et al.* (2010) have studied the biodiversity and abundance of benthic macro-invertebrates community of Kishanpura Lake, Indore. They reported seven species of Oligochaeta like *Tubifex tubifex, Chaetogaster* sp., *Nais simplex, Aeolosoma bengalensis, Dero limosa, Branchiura soverbyi, Stylaria fossularis* and three species of Hirudinea as *Helobdella sp. Glossiphonia sp. and Hemiciepsis marginata* of the family Glossiphonidae. In Gastropoda, three families Planorbidae, Lymnaidae and Viviparidae were recorded. In the family Planorbidae only one species *Planorbis* was identified. However, among family Lymnaidae three species were identified namely, *Limnaea auricularia, L. acumainata* and *other Limnaea sp. Limnaea auricularia* and *L.*

acumainata, whereas in family Viviparidae only three species namely *Vivipara bengalenis, V. oxytropsis* and *Bellamya sp.* were identified. Among Bivalvia (Pelecypoda) only two species of Lamellidens was identified in the family unionidae. Besides this Insects species like *Chironomus phumosus, Strictochironomus sp, Baetis sp., Corixa sp.,Berosus sp., Hydaticus sp. Crustacea Apus (tadpole shrimp), Daphnia (water flea)* was identified during the study period in Kishanpura lake.

Relationship between heavy metals and their impact on aquatic organisms are widely proven. This is due cause by both point and non point anthropogenic activities. Agricultural, industrial and urban effluents are making non point source of contamination. Aquatic organisms including benthic and pelagic species thus contaminated by direct uptake and/or through bioaccumulation. Presence of pollutants and measurement of toxic effects of biological indicators can be used for prediction of expectable toxic influence of known or unknown substances (Gupta and Singh, 2011). Arsenic (As), mercury (Hg), cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), iron (Fe), manganese (Mn), zinc (Zn) etc. are heavy metals, that are not degrade in general and accumulate throughout the ecosystem. This accumulation extends the higher magnitude to lives surrounding the water (Casas *et al.,* 2008). Accumulation of heavy metals are also depends on some other factors like temperature, pH, salinity, age, sex, sexual maturity and other environmental and biological factors (Mubiana, 2006). Monitor of toxic levels in marine organism are need to attention since a long time within food chain and potential risk of human exposure (Giarratano and Amin, 2010). Passive and active monitoring is an approach to monitor bioaccumulation on different levels. Degradation of the ecosystem, elimination of sensitive species and reduction of biodiversity are passive whereas response of artificial or modified populations, behavioral patterns of specimen, specific function of organs like movement, feeding, respiration, reproduction and the neural regulation as well as cellular and sub cellular events are studied under the effect of toxic substances are active monitoring (Solanki *et al.,* 2003). Tissues of organisms are being analyzed to better estimation of bioavailability of metal exposure (Luoma, 1983). Benthic organisms accumulate heavy metals to the levels replicating those in the environment. Concentration of metals in tissues can reflect contamination in molluscs is sensitive bio-monitors of anthropogenic metal inputs (Hendozko *et al.,* 2010). Trace metal makes a serious risk to aquatic organisms. Many researchers already characterized the physiological mechanisms of toxicity in animals exposed to contaminants. Relationships between ambient geochemistry, watershed land-use and trace metal (Cu, Zn, and Pb) concentrations in molluscs, odonates and composite were established (Renier and Sparling, 2001). Aquatic organisms are used as bioindicators for trace metal pollution, in which molluscs are more common. Among other species molluscs are the effective sentinel organisms that can achieve high concentrations of metals and metalloids relative to concentrations ascent of these substances in the surrounding environment (Rainbow *et al.,* 2000). Molluscs and other aquatic organism are biomagnificant and uptake the chemical of water and sediment. Biomagnification can be observed as a distinct case of bioaccumulation in which chemical concentration in organism exceeds in the organism's diet due to dietary absorption. Bioaccumulation is extended and play role in determining water and sediment quality parameters. Assessment of levels of heavy metals pollution in aquatic molluscs are used as bioaccumulation indicators, has become an important duty in preventing risks to public health (Philips, 1997; Zhoua *et al.*, 2008). Gastropods and bivalves molluscs have been accepted as a useful tool for monitoring the environment. They live in such environment because of their ability to accumulate chemical elements and/or compounds in their tissues proportionally to their bioavailability. Thus, it can be used as indicators of aquatic metallic pollution. These are filter feeders, herbivores or carnivores and have potential to bio concentrate water and sediment contaminants even at too low concentrations for detection by routine monitoring techniques. These are sedentary organisms filtrating large amounts of water allowing them to accumulate the substances from environment. They are also satisfying different conditions that can be bioindicators hence very appropriate for monitoring because of their abundance and wide geo- graphical distribution, suitable dimensions, size, weight, relative longer life span, easy identification and collection, abundance in an ecosystem and accumulate the elements to a degree suitable to measure for hazard and risk assessment (Chase *et al.*, 2001; Zhoua *et al.*, 2008). Benthic molluscs are an important bioindicators for heavy metal pollution and are frequently used in global monitoring programs (Rainbow *et al.*, 2000). These are used in both active and passive biomonitoring as well in hazard and risk assessment (Salanki, 1986). Molluscs viz. gastropod mollusc *Bembicium nanum* (Gay and Maher, 2003), *Donax trunculus* and *Chamelea gallina* (Usero *et al.*, 2003), bivalve: *Pyganodon grandis* (Bonneris *et al.*, 2005), *Crassostrea angulata*, *Scrobicularia plana*, *Palameon longirostris*, *Uca tangeri*, *Melicertus kerathurus* (Blasco *et al.*, 1999), *Crassostrea virginica* (Apeti *et al.*, 2005), *Radix ovata* and *Viviparus* spp. (Gundacker, 2000), *Rapana venosa* and *Neverita didyma* (Lee *et al.*, 2006) were used by different researchers from decades for assessing heavy metals in aquatic systems. Molluscs are accumulating most of essential and non-essential heavy metals present in aquatic environment. This is why these organisms are widely used as bioindicator. This is also used in assessment of water quality in coastal and estuarine environments (Gundacker, 2000; de Mora *et al.*, 2004; Nakhle *et al.*, 2006). Different bivalve and gastropod species demonstrated their presence in marine and freshwater riverine ecosystems. They are also suitable for different monitoring projects like trace metals viz. Cd, Cr and Zn in *Perumytilus purpuratus*, *Semelle solida* and *Tagellus dombeii* (Gregori *et al.*, 1996). Mussels can accumulate and integrate concentrations of different metals in seawater for long intervals. They are also assimilating trace metals from their food and ingestion of inorganic particulate material (Philips, 1977). *Mytilus galloprovincialis* (Funes *et al.*, 2006); *Perna perna* (Ferreira *et al.*, 2004) bivalves; *Crassostrea angulata* (Funes *et al.*, 2006); *Crassostrea virginica* (Sokolova *et al.*, 2005) oysters are widely used in laboratory and field studies where uptake, loss or biological effects of heavy metals viz. arsenic, Lead, Cromium, Manganese, Copper, Zinc on east coast of the middle Adriatic Sea (Idris *et al.,* 2007), Black sea (Romeo *et al.,* 2005); Monodonta turbinata and Patella caerulea in Mediterranean area (Campanell *et al.*, 2001), Mytilus edulis, Crassostrea talienwhanensis and Ruditapes philippinarum along with Chinese Bohai Sea (Lee *et al.*, 2006). Some gastropods and oysters exceeded the maximum permissible limit of Cd, Cu and Zn contents in these organisms established by World Health Organisation. *Mytilus trossulus* Barnacle balanus are also used for such metals biomonitoring (Rainbow *et al.*, 2000). Digestive gland of bivalves is the most target organ for accumulation of metals, further, lysosomes of digestive cells are considered as target organelles. Gills of this species have also been shown to accumulate different trace metals in field or in laboratory (Wagner, 2003).

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MATERIALS AND METHODS

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2.1 Materials

2.1.1 Study sites

Sirpur Lake and Bilawali tank of Indore, Madhya Pradesh was taken for this study. Surface water, sediments soil and three species of bivalve viz. *Unio sp*., *Lamellidens corrianus* and *Lamellidens marginalis* were sampled from two different sites of each water bodies.

Sirpur Lake:

This is situated in south west corner of Indore district. This is a gift of Holkar family to city as folklore told. It has 600 acres catchment area covers in Indore Dhar highway, which is a natural habitat for birds. Migratory birds viz. Greater Flamingo and Seniors Crane continue to come on their seasonal visits every year in this Lake area. A diverse flora and fauna makes this lake beautiful and attract visitors. Huge industrialization since from last few decades in nearby area of this Lake makes this Lake polluted. In early 1980s, a renowned photographer Bhalu Mondhe, started restoring the lake with his friend along with an NGO The Nature Volunteers, to restore the lake's ecology and develop it as an ideal birding site (Jain, 2015).Sirpur lake is also recorded as Sirpur Tank and Sirpur tank in different records and literature of government. Even a couple of years before , it was title as tank by Municipal Cooperation.

Sampling site A: The Site A (Sirpur Lake) is located to the East with shore human habitation. Scheme No. 71 industrial area of Indore situated near the catchment area of this site. This is a mix type industrial area having food confectionary industries, printing, plastic, fabrications and some others. This sampling site was situated at 22.702893 East and 75.822562 North (map 1).

Map 1. Sampling site A Sirpur scheme 71 industrial area Indore

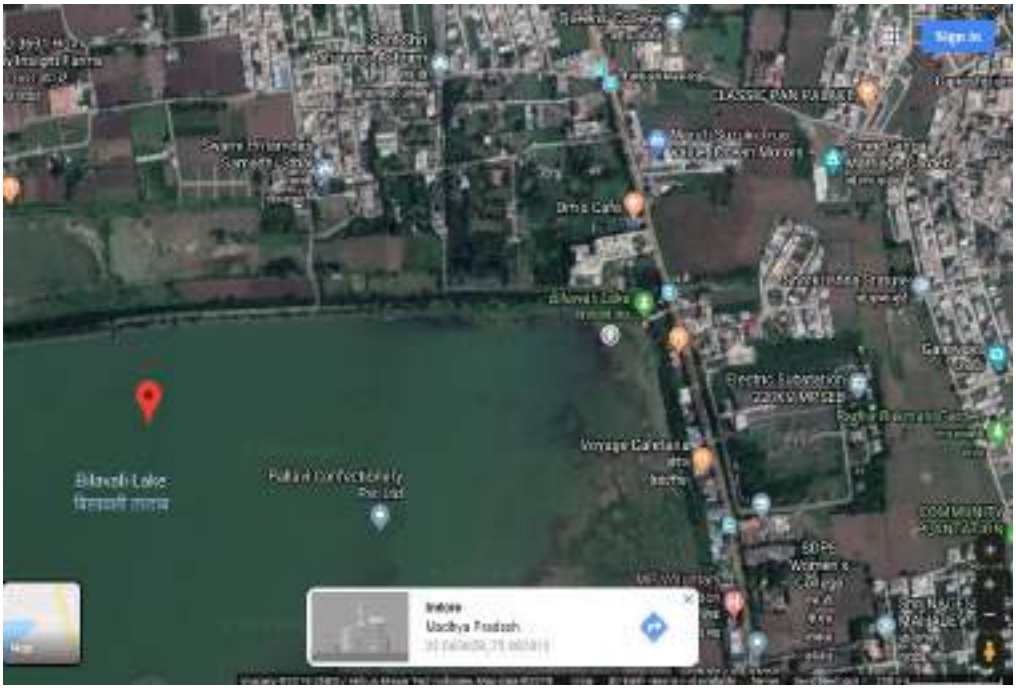
Sampling site B: The Site B (Sirpur Lake) is located to the North with shore human habitation and highway. Sirpur Lake Garden is situated near the catchment area of this site. This sampling site was situated at 22.704056 East and 75.818359 North (map 2).

Map 2. Sampling site B Sirpur Lake Garden Indore

Bilawali tank:

Bilawali tank is situated in southwest direction towards Khandwa road and 6 km. away from Indore, Madhya Pradesh. The catchment area of tank is 117 sq ha. This tank was made by Maharaja Tukoji Rao Holkar under the supervision of Sri Gaddes in 1914. After its completion, the tank was connected to Pipliyapala tank by a canal near the Limbodi village. It is based on the plan of contemporary resident Shri Bhojket in 1905. The tank used for drinking, fish culture agricultures etc.

Sampling site A: The Site A (Bilawali tank) is located to the North with human habitation and food street. Electric substation is situated near to catchment area of this site. This sampling site was situated at 22.663828 East and 75.882010 North (map 3).



Map 3. Sampling site A Bilawali tank food street Indore

Sampling site B: The Site B (Bilawali tank) is located to the West with agricultural land. This sampling site was situated at 22.660956 East and 75.865466 North (map 4).

Map 4. Sampling site A Bilawali tank agricultural land Indore.

2.1.1 Samples and sampling:

Surface water, sediments soil and three species of bivalve viz. *Unio sp*., *Lamellidens corrianus* and *Lamellidens marginalis* were sampled as following:

Water:

Water samples were collected in the summer, moonsoon and winter seasons to establish physico-chemical analysis from both water bodies during June 2016 to May 2017 in triplicate. Sampling was done in morning hours only. The water samples were collected in acid wash plastic bottles from a depth of 5-10 cm below the surface water but rinse thrice when sampling. Water temperature and pH was measured at the time of sampling with the help of thermometer and had held pH meter (Elico LI 120). Water samples were also collected for dissolved oxygen (DO) and heavy metals in a separate 250ml bottle and fixed with alkali reagent.

Soil Sediments:

Soil sediments were collected from two different sampling sites of two Sirpur Lake and Bilawali tank of Indore Madhya Pradesh in summer, monsoon and winter seasons during June 2016 to May 2017 in triplicate. Collected samples were washed and combined with concentrated HNO3 and placed in a 40C fridge prior to laboratory analysis.

Bivalve:

Medium sized 10 specimens of three native species of freshwater bivalve viz. *Lamellidens corrianus*, *Lamellidens marginalis* and *Unio.sp* were collected in summer, monsoon and winter seasons from both water bodies during November 2016 to October 2017. Length and width of 10 animals of each bivalve were measured.

2.2 Methods:

Physico-chemical parameters viz. total alkalinity, dissolved oxygen, total hardness, salinity and chloride; heavy metals viz. Zn, Cu, Pb, Cd, Hg Mn were

determined in laboratory as per standard method of APHA 1998.

Bioaccumulation studies, Oxidative stress viz. Lipid peroxidation (LPO) assay, Assay of glutathione-S-transferees (GST) enzyme activity, Reduced glutathione (GSH) superoxide dismutase (SOD) enzyme activity, Catalase (CAT) enzyme activities and Glutathione peroxidase (GPx) were studied.

Biochemical studies viz. total protein, ascorbic acid, DNA and RNA content in bivalve were also determined and analysed in this study.

Statistical analysis and interpretation was done with the help of Microsoft Excel and Past software.

2.2.1 Physico-chemicals studies

2.2.1.1 Total alkalinity:

Requirements:

Beurate, funnel beaker, beurate stand

Reagents

Phenolphthalein indicator solution: 1gm phenolphthalein indicator was dissolved in 100 ml 95% ethyl alcohol and made volume to 200ml.

Standard hydrochloric acid solution: 2.0ml conc. HCl was dissolved in 200ml distilled water.

Standard sodium carbonate solution (0.045 N): 2.5gm dried sodium carbonate was dissolved in distilled water and made up volume to 1L.

Procedure

200ml water sample was titrated against standard HCl solution in the presence of phenolphthalein indicator. End point was noted at the point of disappearance of pink colour.

Calculation

Alkalinity (mg$CaCO_3$/L= A x B x 50,000 / ml sample

Where,

A = ml titrant,

B = Normality of titrant

2.2.1.2 Dissolved oxygen:

Requirements:

Beurate, stand, funnel, BOD bottles, glassware etc.

Reagents:

Manganous sulfate solution: MnSO4.H2O 36.4gm has been dissolved and filtered into distilled water. Filtrate was then combined with distilled water and produces volume up to 100 ml. Solution was checked with starch.

Alkali iodide azide reagent: 1gm. sodium azide was dissolved in 50 ml of distilled water to form solution A. 48gm of sodium hydroxide and 75gm of sodium iodide were separately dissolved into distilled water to form solution B, consequently all solutions A and B were combined and volume to 100 ml.

Conc. H_2SO_4:

Starch indicator solution: 50mg soluble starch powder was dissolved in 80ml hot distilled water and was make volume with distilled water to 100ml.

Standard sodium thio sulfate solution (0.025N): 1.241gm Na2S2O3.5H2O was dissolved in distilled water freshly boiled and cooled. Added 80 mg strong NaOH and then added 200ml of distilled water to final volume. Standard potassium bi-iodate solution (0.025N): 812.4mg potassium bi-iodate was dissolved in distilled water and make volume to 1L.

Procedure:

300ml sample was taken in a bottleA portion replacement of the cap added 1ml of manganese sulfate and alkali iodide azide solution. Bottle was shaken 10 times and brown precipitation was then allowed to settle. 2ml. Concentrated H2SO4 was then applied to the precipitate dissolving container, resulting in a yellow solution at full dissolution.20ml of yellow solution obtained was titrated against regular hypo-tonic solution in the presence of starch indicator. The end state was marked by the blue color disappearance

Calculation:

1ml standard hypo solution of 0.025 N = 200μg dissolved oxygen.

2.2.1.3 Total hardness:

Requirements:

Beurate, stand, funnel, bottles, glassware etc.

Reagents:

Sodium hydroxide (6N): 24gm NaOH was dissolved in distilled water and volume made up to 100ml.

Murexide indicator (Ammonium perpurate): 200mg murexide powder was grinded in 100gm anhydrous sodium chloride.

Standard EDTA (0.01M): 3.723gm EDTA was dissolved to made volume up to 1L and standardized against standard calcium solution.

Standard calcium solution: 1gm $CaCO_3$ was dissolved in HCl : Water (1:1). 200ml distilled water was then added, boiled and cooled. 2 drop methyl red indicator was mixed and intermediate orange colour was adjusted with 1: 1 HCl : Water solution. This solution was then diluted in distilled water and made volume to 1L. This was equivalent to 1mg $CaCO_3$/ml.

Conc. HNO_3

Conc. HCl

Conc. H_2SO_4

Procedure:

Pretreatment

100ml sample water was acidified with diluted HNO_3 and H_2SO_4. Stand to evaporate till white residue. Obtained white residue was dissolved in 1:1 HCl:Water, neutralized with conc. NH_4OH and make volume up to 100ml with distilled water.

Titration

50ml pretreated sample was taken in a beaker and 4ml 6N NaOH was added to raise pH up to 13. 0.2gm murexide indicator was added. Mixture was hen titrate against EDTA solution. End point of titration was observed till pink to purple. Titration value was calculated as following:

Calcium hardness (mg/L) = (A x B x 1000) / ml sample

Where,

A = ml EDTA titrant

B = mg $CaCO_3$ equivalent to 1 ml EDTA solution

2.2.1.4 Salinity:

Electrical conductivity was measured through Systronics (Degree of accuracy 0.01) equipped with an EC probe.

2.2.1.5 Chloride:

Requirements:

Beurate, funnel beaker, beurate stand

Reagents:

Potassium chromate indicator solution: 5 gm potassium chromate was dissolved in little amount of distilled water. Silver nitrate solution was then added till the formation of red precipitate. The solution was allowed to stand for 12 hours and then filtered. Volume was makeup to 100ml by distilled water.

Standard silver nitrate titrant (0.0141N): 2.395 gm $AgNO_3$ was dissolved in distilled water and diluted to 1 liter. Solution was standardized against standard sodium chloride solution.

Standard sodium chloride solution (0.0141 N):824.0mg NaCl (dried at

140°C) was dissolved in chloride free diluted water and make the volume to 1 liter (1 ml of standard solution = 500 μgm Cl^{-}).

Procedure:

100 ml sample was titrated against the silver nitrate solution in the presence of potassium chromate indicator. End point of titration was indicated by appearance of pinkish yellow colour of silver chromate. A blank was also titrated simultaneously, and the obtained values were computed in the formula.

Calculation:

Chloride mg/L = (A - B) x N x 35,450 ml sample

Where,

N = Normality of titrant.

A = ml titrant used for sample,

B = ml titrant used for blank,

2.2.1.6 Temperature and pH

Temperature and pH was determined at the sampling sites with the help of respective probes and digital meter.

2.2.2 Determination of heavy metal levels:

Sediment soil sample preparation:

The gathered soil samples were sealed using a sieve (mesh size 0.5 mm) and the fine fractions extracted were air-dried. Air-dried samples were then grounded using a glass mortar and thoroughly sieved again and homogeneous powders were eventually collected, were preserved in a desiccators. 5gm oven dried sieved powder sample of soil sediments was taken in a conical flask. Then about 20 ml of concentrated HCL was then added. The sample digestion was performed on a hot plate by putting watch glass for about 1 hour on each conical flask and evaporating to around dryness, the digested sample was ready for analysis (Ahmed et al., 2002).

Preparation of bivalve soft tissues for heavy metal analysis:

The sampled bivalves were dissected within 12 hours of acquisition and their entire soft body tissues were cut, washed in distilled water and afterwards separately dried in the oven at around 700-800 C. After oven drying dry weight of the tissue was measured. The tissues were powdered and stored separately by labeling the specimen with date, species name and sampling site name. 500mg dry powders of whole soft body tissue of bivalves were digested in 10ml mixture of Nitric acid: Perchloric acid in the ratio of 5:1. The samples were left overnight after half an hour of stirring and were digested on hot plate until the clear white fumes appeared. The solution volume of 10ml was created by adding a mixture of nitric acid and perchloric acid phase by phase. Double glass distilled water was added after leaving the flask to cool to bring the volume to 50 ml in volumetric flask and finally solution was filtered via Whatman filter paper number 41. Similar processes were also extended to surface water and soil sediments.

Heavy metals analysis:

The Atomic Absorption Spectrophotometer (AAS) was used for heavy metal concentrations in surface water, soil sediments and whole soft body tissue of bivalves. (Thermo Scientific, U. K. make, Solaar A series model). Flame temperature of air–acetylene used for estimation of Zn, Cd, Mn,Hg Cu and Pb was 1100^{o}C, 1000^{o}C, 2100^{o}C and 1200^{o}C respectively. While band path for Zn Hg and Mn was 0.2 and for Cd, Cu and Pb was 0.5. Before each metal determination the AAS was calibrated for each metal using lower detection limit of AAS was 0.004mg/L for Zn and Cd,0.005mg/L for Cu and 0.003mg/L for Pb and Hg , 0.002mg/L for Mn. All reagents used were A.R. grade (Merck). To prevent contamination, all test equipment washed in 10% HNO3 solution and rinsed with distilled water before use. After every 10 samples, procedural blanks and quality control samples prepared from Zn, Cu, Hg , Mn Cd and Pb solutions were analyzed to check the accuracy of the samples. For the measurement of the metal concentration per unit body weight (μg / g) and metal body burden (μg / individual), dry weight of each animal was used. Results are presented as in mean ± standard deviation (SD). All results are presented on a dry weight basis as μg/gm for heavy metal concentration.

2.2.3 Metal bioaccumulation studies:

To trace the efficiency of metal bioaccumulation in bivalve species, Biowater Accumulation Factor (BWAF) and Biosediment Accumulation Factor (BSAF) was calculated. The BWAF factor is specified as the ratio of metal concentration in the organism to that in water. The BSAF is defined as the ratio of metal concentration in the organism with that in the sediments. (Usero *et al.,* 2005; Szefer *et al.,* 1999).

BWAF = Concentration of heavy metals in animal tissue / Concentration of heavy metals in water

BSAF = Concentration of heavy metals in animal tissue / Concentration of heavy metals in soil sample

2.2.4 Oxidative stress (Biomarkers) studies:

Sample preparation:

Digestive glands of five animals of each species was collected, separated, surface blotted with tissues paper and thoroughly washed with phosphate buffer (50mM; pH 7.4). 1gm of blotted tissue was homogenized with 50mM phosphate buffer pH 7.4 at 4^0C and centrifuged at 10000 g for 20 min at 4^0C. Obtained supernatant was used for biochemical analysis viz. LPO, GSH, SOD, CAT, GPx and GST.

I) Lipid peroxidation (LPO) assay:

The method of Oshkawa et al., (1979) was used to determined lipid peroxidation (LPO). Tissue homogenate was prepared in 5% TCA (100 mg tissue/ml). 4 ml of 0.5% TBA in 20% TCA was added in 1ml homogenate tissue and incubated at 95^0C for 30 minutes. This was instantly frozen in ice which changed color from orange to pink. The sample obtained was centrifuged at 4000 rpm for 10 minutes, and supernatant absorption was taken at 532 (specific) and 600 nm (non-specific absorption).Lipid peroxidation (MDA) formed was calculated by using the following formula:

Amount of MDA formed (µmoles/ml) = $((A_{532}-A_{600})/155)+(V/v)$

Where,
A_{532} = Absorbance at 532nm
A_{600} = Absorbance at 600nm
V = Total volume of assay mixture (5ml)
V = Volume of sample (1ml)

II) Assay of glutathione-S-transferees (GST) enzyme activity:

Glutathione-S-transferees (GST) activity was estimated by the method of Habig *et al.*, (1974). 0.5gm sample was homogenized with 5ml of phosphate buffer and centrifuged at 10,000 rpm for 15 minutes at 4^0C. Supernatant was used for the assay. A sample mixture contained 0.1ml of GSH, 0.1ml of CDNB, and a total volume of 2.9 ml of phosphate buffer. The reaction was triggered by enzyme extract adding 0.1ml. During three minutes, readings were taken at 340 nm every 15 seconds against distilled water blank using double beam spectrophotometer. The activity of Glutathione-S-transferees (GST) was measured using the extinction coefficient of the formulated product (9.6 mM-1cm-1) and presented as nmoles of CDNB conjugated / min / mg protein. Glutathione-S-transferees (GST) activity was calculated by using the following formula:

$$\Delta 340/min=((A_{340}(Time\ 2))-(A_{340}\ (Time\ 1)))/(Time\ 2\ (min)-Time\ 1\ (min))$$

III) Reduced glutathione (GSH) assay for ROS:

The content of reduced glutathione (GSH) in the samples was acessed by the method carried by Boyne and Ellman (1972). 1ml of tissues extracts was fed with 4.0 ml of metaphosphoric acid precipitating solution (1.67 g of glacial meta phosphoric acid, 0.2g EDTA and 30 g NaCl dissolved in 100 ml water). After centrifugation, 0.2 ml of the protein free supernatant was mixes with 0.2 ml of 0.4 M Na_2HPO_4 and 1.0 ml of DTNB reagent (40 mg of DTNB in 100 ml of aqueous 1% tri sodium citrateAbsorbance was read within 2 minutes using a double beam spectrophotometer (Elico BL 200), at 412 nm. Low concentrations of glutathione (GSH) were expressed as nmol / mg protein. Reduced glutathione (GSH) level was calculated by using the following formula:

Conc. of sample (µl/ml) = (Sample OD x Conc. of std.) / OD std mg/gm wet tissue = Conc. of sample/ wet tissue in gm

Where,

Conc. of std = concentration of standard

OD std = Optical density of standard

IV Measurement of superoxide dismutase (SOD) enzyme activity:

Superoxide dismutase (SOD) activity was estimated by the method of Beauchamp and Fridovich (1973).

Assay mixture:

Component	Amount added		Final
	Control	Sample	concentration
Phosphate buffer pH 7.8 (100mM)	1.5ml	1.5ml	50mM
Methionine (65mM)	0.6ml	0.6ml	13mM
Distilled water	0.560ml	0.510ml	------
EDTA	0.001ml	0.001ml	------
Enzyme extract	------	0.05ml	------
NBT (750µl)	0.3ml	0.3ml	0.07µM
Total	3.261ml	3.261ml	------

To drive out other metal ions, EDTA was added. Last added riboflavin, and shaken tubes. One set of tubes was illuminated for 30 min. at a distance of 1 cm under light source and another set of tubes was kept for 30 min. in the dark. The control was the concentrate held under light as well as in dark, without the enzyme extract. Superoxide dismutase (SOD) operation was achieved using spectrophotometer with double beam. Enzyme activity was calculated by using the following formula-

Enzyme units (Units/ml) = c – (E/ (C/2)) x (Total amount of assay mixture / Amount of enzyme extract added)

Where,

C = the difference in absorbance read at 560 nm of the control tubes kept for incubation in light and dark.

E = the difference in absorbance read at 560 nm of the tubes with the enzyme kept for incubation in light and dark.

IV) Measurement of catalase (CAT) enzyme activity:

Catalase (CAT) is calculated by (Aebi, 1984) using hydrogen peroxide as a substrate.

Component	Volume	Final concentration
0.1 M Phosphate buffer	1.0 ml	0.1 M

30% H_2O_2 (1:10 diluted)	10 µl	3%
Enzyme extract	50 µl	----
Total	1.06 ml	----

Phosphate buffer and enzyme extract were applied to 1 ml quartz cuvettes and the reaction began with the addition of H2O2 and the absorbance was read continuously for 3 minutes at 240 nm using a double beam spectrophotometer at 30 seconds interval. One unit of enzyme is represented as the quantity of enzyme required to convert 1 mole substratum (H2O2) into a product (H2O+O2) in one second. Catalytic Units of Enzyme (b) were calculated using the formula:

$b = ((\Delta A \text{ x } V \text{ x } 1000) / (\varepsilon \text{ x } d \text{ x } \Delta t \text{ x } v))\mu\text{moles x min}^{-1} \text{ x } 1^{-1} = U/I$

Where,

$\Delta A / \Delta t$ = Slope (Slope is calculated by plotting a graph of time v/s absorbance at 240 nm)

V = total volume of the assay mixture (1.06 ml) V = volume of sample in assay mixture (50 µl) d = path length=1cm

ε = Extinction coefficient of H_2O_2 = 0.003942 l x mM^{-1}x mm^{-}

V) Measurement of Glutathione peroxidase (GPx) enzyme activity:

Glutathione peroxidase (GPx) was obtained using Rotruck et al. (1973) procedure with some modifications. 0.4ml of 0.4M sodium phosphate buffer (pH 7.0), 0.1ml of 10mM sodium azide, 0.2ml of 4mM reduced glutathione, 0.1ml of 2.5mM H_2O_2, 0.2ml of distilled water and 0.5ml of enzyme was mixed and incubated at 0, 30, 60, 90 seconds respectively. The reaction was terminated with 0.5ml of 10% TCA and 2ml of the supernatant was applied to 3ml of phosphate buffer and 1ml of DTNB reagent (0.04% DTNB in 1% sodium citrate) after centrifugation. Optical density was measured using a double beam spectrophotometer at 412 nm. Glutathione peroxidase (GPx) function is expressed in terms of µg / min / mg protein used for glutathione. Glutathione peroxidase (GPx) activity was calculated by using the following formula:

z = OD30 –OD 90 = z/ factor

b= 1000 x (a/500) [for 500µl/0.5ml]

Therefore,

1ml =1000 x (b/500) = c per mg of protein = c/total amount of protein

Where,

OD 90 = Optical density at 90 seconds

OD 30 = Optical density at 30 seconds

Factor = 0.0436

2.2.5 Biochemical Studies:

I) Whole soft tissues of the body were removed and dried in the oven at 700 to 800C until the constant weight of the dry tissues was reached. The content of proteins, ascorbic acid RNA and DNA was measured according to the following methods:

II) Protein estimation

The tissue protein content was calculated using Lowry's (Lowry et al., 1951) method. Every tissue was homogenized by 10 mg of dry powder in a small amount of 10% TCA and diluted by 10% TCA to 10 ml.. Then it was centrifuged for 15 minutes at 3000 rpm. The supernatant was used for ascorbic acid estimation. At the bottom of centrifuged tubes the protein precipitate was dissolved in a 10 ml 1.0 N NaOH solution. 0.1 ml of this solution was added to each powder in three test tubes containing 0.9 ml of distilled water and 4.0 ml of freshly prepared Lowry's ' C '. After adding 0.5 ml Folin’s phenol reagent, the test tubes were incubated in dark for 30 minutes at 37^0C. The optical density of blue colour developed was read at 530nm on a double beam Spectrophotometer. The blank was prepared in the same way without dissolved precipitate protein. By referring to the standard graph value, the protein content in different tissues was measured and expressed in terms of dry tissue mg protein/100 mg. The standard use of the Bovine serum albumen.

Ascorbic acid estimation

Estimation of ascorbic acid was carried out by the method of Roe (1967). 1.0ml supernatant was extracted from homogeneous test tubes which were already centrifuged to estimate protein. In these test tubes 0.25 ml hydrazine reagent aliquot was added. The reaction mixture was kept in boiling bath water for 15 minutes. It has been cooled and 85% H2SO4 has been applied to 3.0 ml ice cold with regular stirring. The reaction mixture was kept at normal room temperature for 30 minutes. Optical density was read at 530 nm on a double beam Spectrophotometer. 1.0 ml 10% TCA was used as a blank, while ascorbic acid was used as a standard. The quantity of ascorbic acid content was determined by using standard graph values in different tissues. Ascorbic acid was measured as mg per 100 mg of dry tissue.

Ribonucleic acid (RNA) estimation

RNA content was measured using the Volkin and Cohn Orcinol (1954) method. Homogenized 10 mg of dry tissue powder by adding 10 ml of distilled water. It was then centrifuged to 3000 rpm for 10 minutes. Gathered the supernatant containing RNA. After taking 1ml of supernatant and inserting 3ml of Orcinol reagent. The solution was then put in the test tube for 15 minutes in a boiling water bath and then cooled. Next, a double beam spectrophotometer (Elico BL 200) read the optical density of the RNA at 665 nm.

Orcinol reagent was used as a blank. RNA content in the tissue was measured using different concentrations of pure RNA powder with the same reagent and technique from the calibration curve obtained.

Results are expressed as mean ± standard deviation (S.D.). The ANOVA test was used to see if biochemical components differ significantly between reservoirs seasons and bivalve organisms. The probabilities were considered statistically significant, less than 0.05 ($p<0.05$). All statistical analyses were carried out with version SPSS 21.0.

Deoxyribonucleic acid (DNA) estimation

The content of tissue DNA was calculated using Burton's Diphenylamine (1956) system. By adding 10 ml distilled water, 10 mg of dry tissue powder was homogenised. Then mixture was centrifuged for 10 minutes at 3000 rpm. The DNA-containing centrifuged was collected. After taking 1ml of supernatant and adding 3ml of diphenylamine reagent. The solution was then put in the test tube for 10 minutes in the boiling bath of water. It had been allowed to cool after boiling the solution in the test tube. Then on a double beam spectrophotometer, the DNA's optical density was read at 595 nm. Diphenylamine was used as blank reagent. The DNA content in the tissue was measured using different concentrations of pure DNA powder with the same reagent and procedure from the calibration curve obtained.

RESULTS

RESULTS

Physico-chemical analysis of surface water and heavy metal determination in water and soil sediments of Sirpur lake and Bilawali tank of Indore was studied. Dry weight whole body; metal concentration per unit body weight (µg/g); metal Body burden (µg/individual); BWAF (Bio-water accumulation factor); BSAF (Bio-sediment accumulation factor); protein content, ascorbic acid content and DNA content in gills, digestive gland and whole soft body in of three species of bivalves viz. *Lamellidens corrianus*, *Lamellidens marginali* and *Unio* sp. collected from Sirpur lake and Bilawali tank, Indore were studied to determine effect of pollution on such species in this study.

3.1 Physico-chemical studies of water samples:

Physico-chemical parameters viz. total alkalinity, dissolved oxygen, total hardness, salinity and chloride in water samples were determined seasonally in water samples collected from Site A (Sirpur Lake) located near Scheme No. 71 industrial area of Indore, Site B (Sirpur Lake) located near Sirpur Lake Garden, Indore, Site A (Bilawali Tank) located near food street Bilawali Tank, Indore and Site B (Bilawali tank) located to the West of Bilawali Tank agricultural land. Table 1 represents the mean with standard deviation of collected observations.

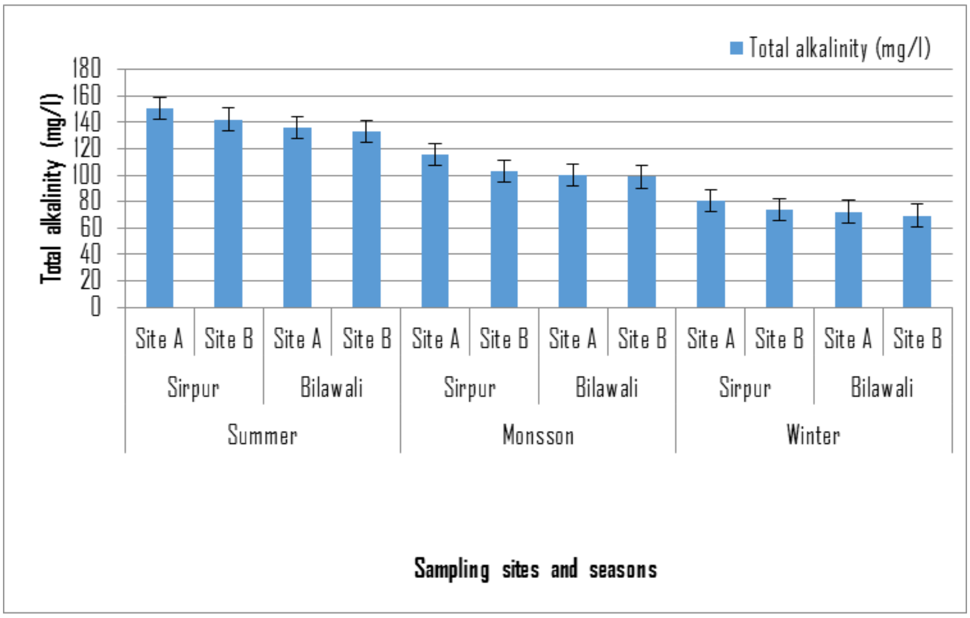
Table 1. Seasonal variations in physico-chemical parameters of water samples collected from Sirpur and Bilawali Tanks of Indore.

	Summer				**Monson**				**Winter**			
	Sirpur		**Bilawali**		**Sirpur**		**Bilawali**		**Sirpur**		**Bilawali**	
Parameters	**Site A**	**Site B**	**Site A**	**Site B**	**Site A**	**Site B**	**Site A**	**Site B**	**Site A**	**Site B**	**Site A**	**Site B**
Total alkalinity (mg/l)	150.29±2.12	142.35±2.46	136.42±2.33	133.14±2.96	115.55±1.86	103.22±2.54	100.25±2.46	98.72±1.46	80.95±2.10	74.12±1.96	72.4±2.45	69.42±3.10
Dissolved oxygen (mg/l)	6.81±0.42	6.87±0.51	7.12±0.68	7.46±0.55	7.41±0.68	7.95±0.48	7.96±0.35	8.01±0.76	9.26±0.63	9.68±0.56	9.84±0.46	9.12±0.45
Total hardness (mg/l)	166.35±6.21	153.46±6.78	148.65±7.25	140.12±5.86	112.5±6.02	98.65±4.23	97.02±3.95	95.96±4.12	138.62±5.42	126.42±4.56	120.45±5.12	117.25±2.56
Salinity (mg/l)	113.9±2.34	106.92±2.10	102.25±2.42	97.56±2.01	71.54±2.34	67.24±2.10	63.12±2.46	57.24±2.34	108.25±2.54	97.12±3.12	93.56±4.23	91.35±3.15
Chloride (mg/l)	63.4±1.51	60.35±1.69	57.14±1.78	54.2±1.64	41.3±1.56	37.51±1.42	34.95±1.94	32.01±2.01	60.01±1.95	54.1±1.56	52.25±1.45	51.02±2.01
Temperature (°C)	28.75±1.33	27.85±2.01	27.96±2.36	27.25±2.23	23.56±2.03	24.35±2.65	24.55±2.84	24.95±2.56	21.24±2.15	21.02±2.59	21.35±2.56	21.56±2.30
pH (unit)	7.9±0.72	8.01±0.74	7.82±0.62	7.61±0.60	8.04±0.58	7.7±0.75	7.45±0.56	7.34±0.48	7.69±0.76	7.24±0.56	7.06±0.49	7.01±0.72

3.1.1 Total alkalinity:

A graphical representation of total alkalinity with standard error in collected water samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 1. Total alkalinity with standard deviation in water samples collected from Sirpur Lake site A and site B were 150.29±2.12 and 142.35±2.46 in summer; 115.55±1.86 and 103.22±2.54 in monsoon; 80.95±2.10 and 74.12±1.96 in winter seasons whereas, Bilawali Tank site A and site B were reported 136.42±2.33 and 133.14±2.96 in summer; 100.25±2.46 and 98.72±1.46 in monsoon; 72.4±2.45 and 69.42±3.10 in winter seasons respectively.

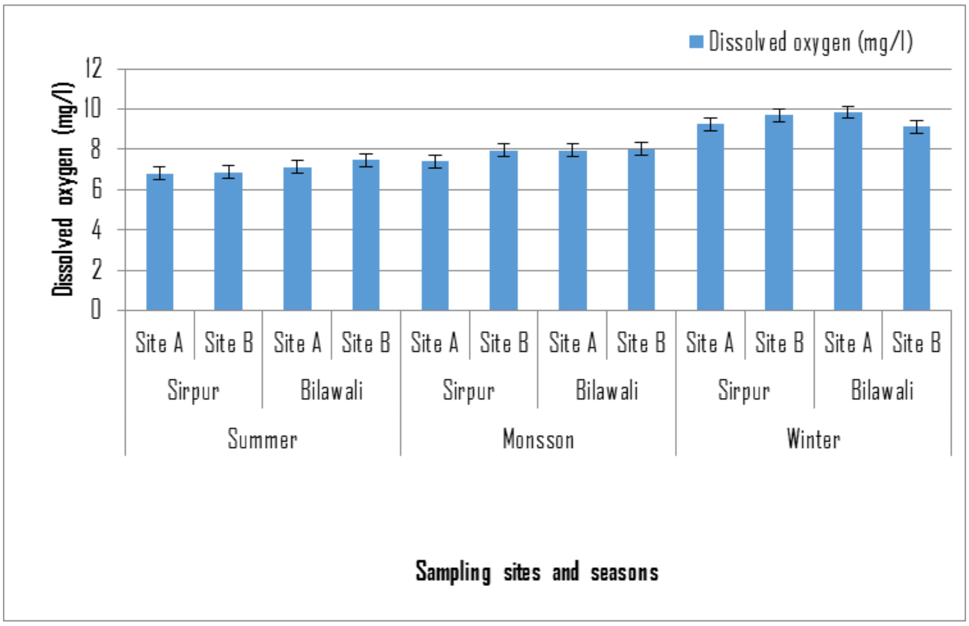
Fig. 1. Total alkalinity in water samples collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.1.2 Dissolved oxygen:

Dissolved oxygen with standard error in collected water samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 1. Dissolved oxygen with standard deviation in water samples collected from Sirpur Lake site A and site B were 6.81±0.42 and 6.87±0.51 in summer; 7.41±0.68 and 7.95±0.48 in monsoon; 9.26±0.63 and 9.68±0.56 in winter seasons whereas, Bilawali Tank site A and site B were reported 7.12±0.68 and 7.46±0.55 in summer; 7.96±0.35 and 8.01±0.76 in monsoon; 9.84±0.46 and 9.12±0.45 in winter seasons respectively.

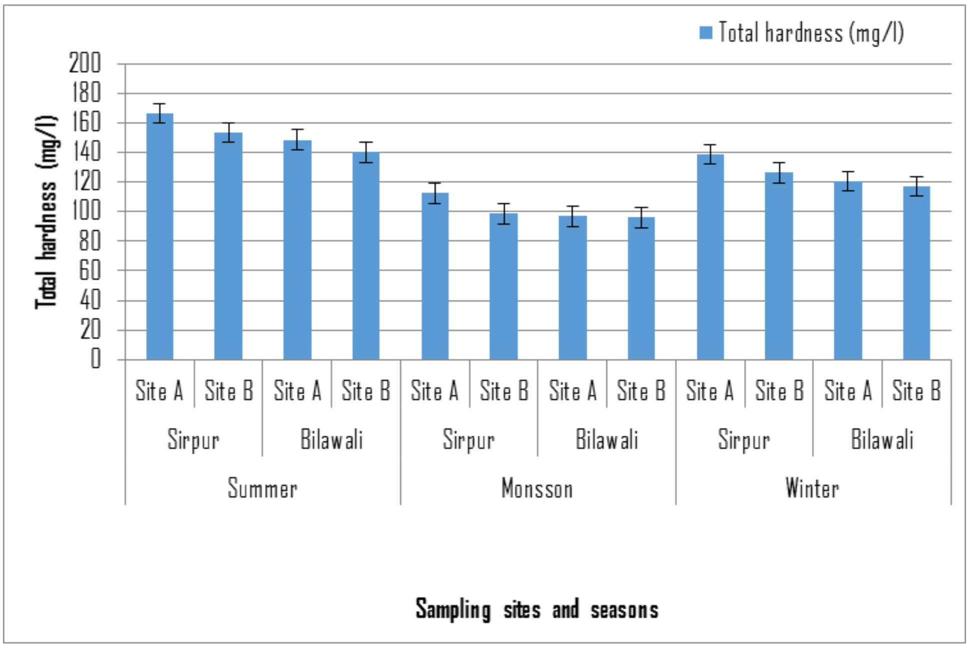
Fig. 2. Dissolved oxygen in water samples collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.1.3 Total hardness:

A graphical representation of total hardness with standard error in collected water samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 1. Total hardness with standard deviation in water samples collected from Sirpur Lake site A and site B were 166.35±6.21 and 153.46±6.78 in summer; 112.5±6.02 and 98.65±4.23 in monsoon; 138.62±5.42 and 126.42±4.56 in winter seasons whereas, Bilawali Tank site A and site B were reported 148.65±7.25 and 140.12±5.86 in summer; 97.02±3.95 and 95.96±4.12 in monsoon; 120.45±5.12 and 117.25±2.56 in winter seasons respectively.

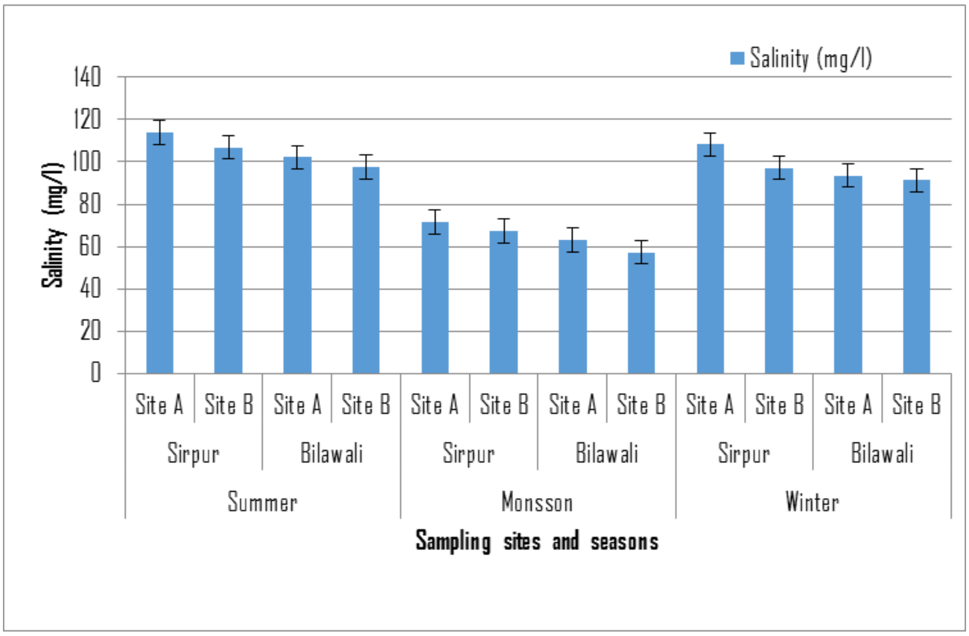
Fig. 3. Total hardness in water samples collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.1.4 Salinity:

Salinity with standard error in collected water samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 1. Salinity with standard deviation in water samples collected from Sirpur Lake site A and site B were 113.9±2.34 and 106.92±2.10 in summer; 71.54±2.34 and 67.24±2.10 in monsoon; 108.25±2.54 and 97.12±3.12 in winter seasons whereas, Bilawali Tank site A and site B were reported 102.25±2.42 and 97.56±2.01 in summer; 63.12±2.46 and 57.24±2.34 in monsoon; 93.56±4.23 and 91.35±3.15 in winter seasons respectively.

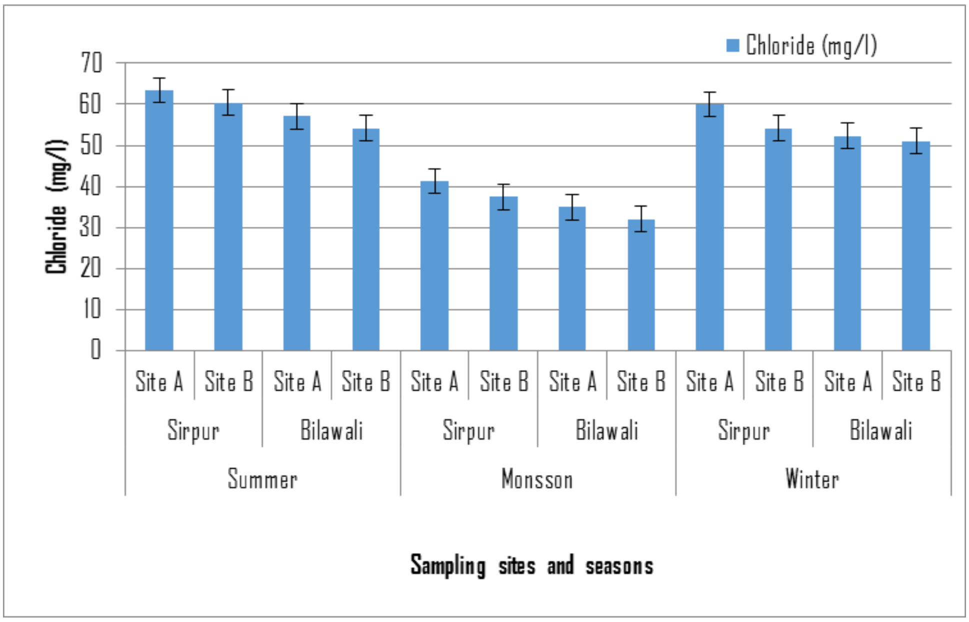
Fig. 4. Salinity in water samples collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.1.5 Chloride:

A graphical representation of chloride with standard error in collected water samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 1. Chloride with standard deviation in water samples collected from Sirpur Lake site A and site B were 63.4±1.51 and 60.35±1.69 in summer; 41.3±1.56 and 37.51±1.42in monsoon; 60.01±1.95 and 54.1±1.56 in winter seasons whereas, Bilawali Tank site A and site B were reported 57.14±1.78 and 54.2±1.64 in summer; 34.95±1.94 and 32.01±2.01 in monsoon; 52.25±1.45 and 51.02±2.01 in winter seasons respectively.

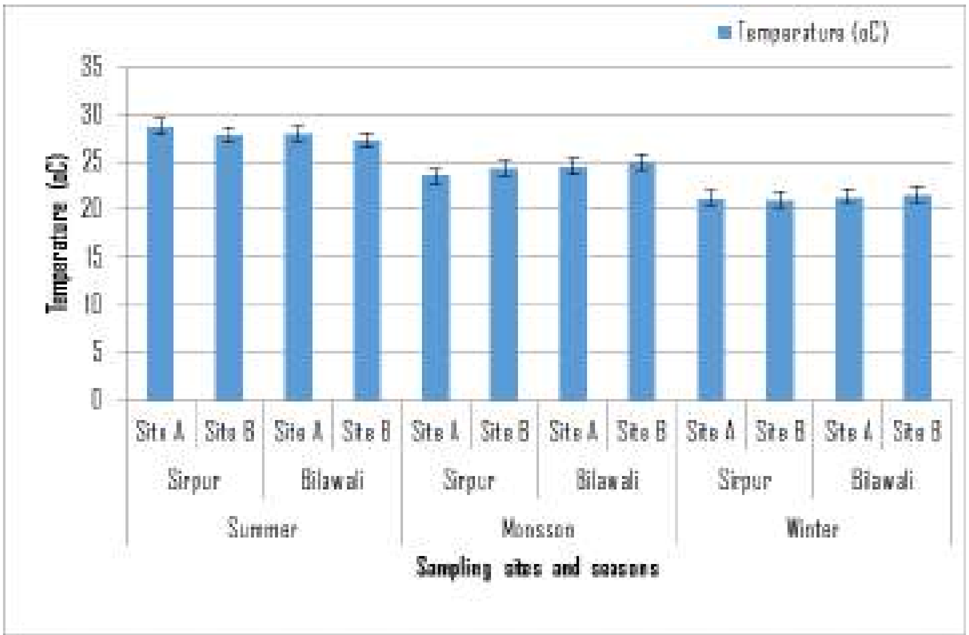
Fig. 5. Chloride in water samples collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.1.6 Temperature:

Temperature with standard error in collected water samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 1. Temperature with standard deviation in water samples collected from Sirpur Lake site A and site B were 28.75±1.33 and 27.85±2.01 in summer; 23.56±2.03 and 24.35±2.65 in monsoon; 21.24±2.15 and 21.02±2.59 in winter seasons whereas, Bilawali Tank site A and site B were reported 27.96±2.36 and 27.25±2.23 in summer; 24.55±2.84 and 24.95±2.56 in monsoon; 21.35±2.56 and 21.56±2.30 in winter seasons respectively.

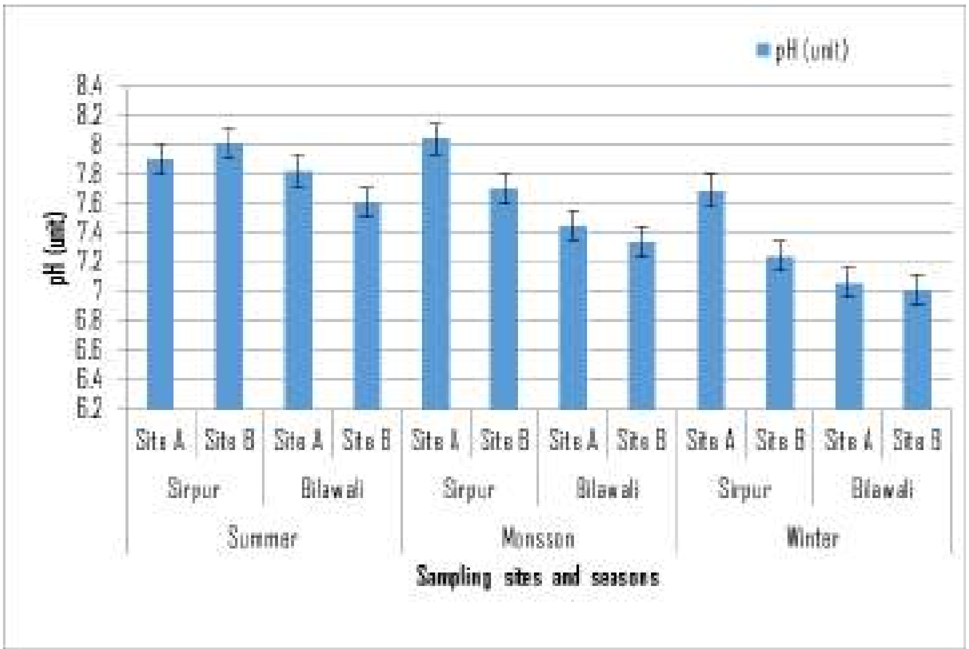
Fig. 6. Water temperature of collected water samples of different sampling sites of Sirpur and Bilawali Tanks of Indore in different season.



3.1.7 pH:

A graphical representation of pH with standard error in collected water samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 1. pH with standard deviation in water samples collected from Sirpur Lake site A and site B were 7.9±0.72 and 8.01±0.74 in summer; 8.04±0.58 and 7.7±0.75 in monsoon; 7.69±0.76 and 7.24±0.56 in winter seasons whereas, Bilawali Tank site A and site B were reported 7.82±0.62 and 7.61±0.60 in summer; 7.45±0.56 and 7.34±0.48 in monsoon; 7.06±0.49 and 7.01±0.72 in winter seasons respectively.

Fig. 7. Water pH of collected water samples of different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.2 Studies of heavy metals in water and soil sediment samples:

Heavy metals viz. Zn, Cu, Mn Hg, Pb and Cd in water and soil sediment samples collected from Site A (Sirpur Lake) located near Scheme No. 71 industrial area of Indore, Site B (Sirpur Lake) located near Sirpur Lake Garden, Indore, Site A (Bilawali Tank) located near food street Bilawali Tank, Indore and Site B (Bilawali tank) located to the West of Bilawali Tank agricultural land were determined seasonally.

3.2.1 Heavy metals in water sample:

Table 2 presents the mean with standard deviation of collected observations of heavy metals in water samples.

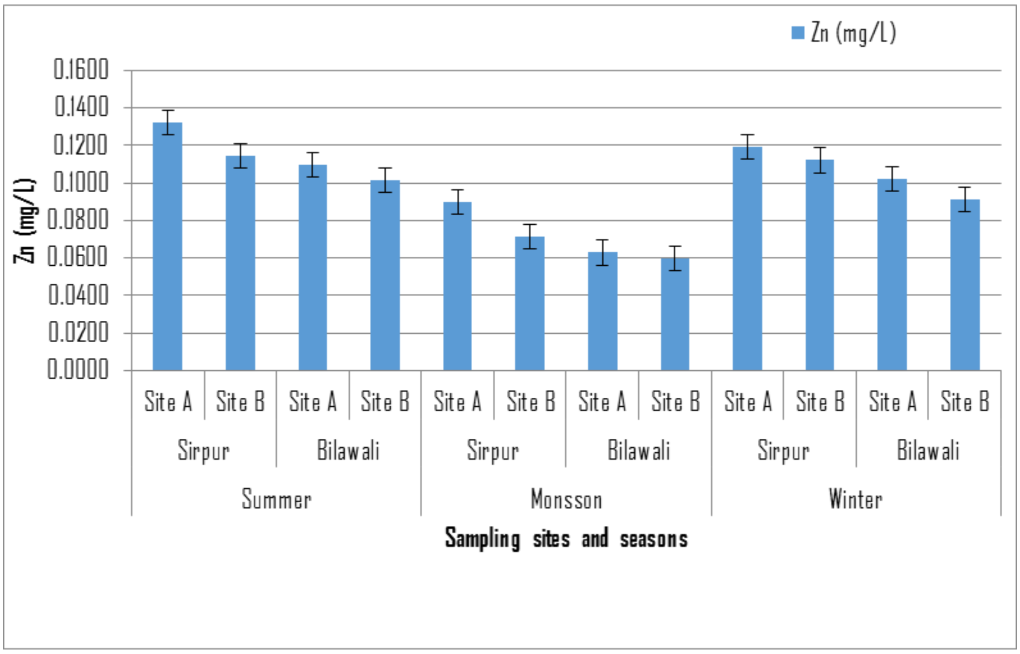
Table 2. Seasonal variations in presence of heavy metals in water samples collected from Sirpur and Bilawali Tanks of Indore.

Heavy metals in surface water	**Summer**				**Monsson**				**Winter**			
	Sirpur		**Bilawali**		**Sirpur**		**Bilawali**		**Sirpur**		**Bilawali**	
	Site A	Site B	Site A	Site B	Site A	Site B	Site A	Site B	Site A	Site B	Site A	Site B
Zn (mg/L)	0.1320± 0.0008	0.1145± 0.0006	0.1095± 0.0007	0.1012± 0.0006	0.0897± 0.0006	0.0712± 0.0006	0.0628± 0.0006	0.0598± 0.0004	0.119± 0.0007	0.1121± 0.0008	0.1022± 0.0008	0.0912± 0.0005
Cu (mg/L)	0.0246± 0.0004	0.021± 0.0007	0.0198± 0.0006	0.0196± 0.0005	0.0167± 0.0006	0.0149± 0.0006	0.0138± 0.0007	0.0114± 0.0003	0.0149± 0.0008	0.0159± 0.0008	0.0151± 0.0009	0.0152± 0.0002
Pb (mg/L)	0.0301± 0.0005	0.0296± 0.0004	0.0256± 0.0004	0.0241± 0.0004	0.0268± 0.0004	0.048± 0.0004	0.0221± 0.0005	0.0194± 0.0003	0.0201± 0.0009	0.0243± 0.0007	0.0221± 0.0009	0.0221± 0.0003
Cd (mg/L)	0.0086± 0.0001	0.0079± 0.0002	0.0072± 0.0002	0.0067± 0.0001	0.0072± 0.0003	0.0069± 0.0002	0.0067± 0.0002	0.0057± 0.0002	0.0076± 0.0002	0.0069± 0.0003	0.0071± 0.0003	0.0069± 0.0001
Hg (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mn (mg/L)	0.0102± 0.0002	0.0090± 0.0001	0.0081± 0.0001	0.0073± 0.0001	0.0092± 0.0002	0.0076± 0.0001	0.0075± 0.0001	0.0062± 0.0001	0.0079± 0.0001	0.0068± 0.0001	0.0069± 0.0001	0.0062± 0.0001

3.2.1.1 Zinc (Zn)

Concentration of Zinc with standard error in collected water samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 2. Concentration of Zinc with standard deviation in water samples collected from Sirpur Lake site A and site B were 0.1320±0.0008 and 0.1145±0.0006 in summer; 0.0897±0.0006 and 0.0712±0.0006 in monsoon; 0.119±0.0007 and 0.1121±0.0008 in winter seasons whereas, Bilawali Tank site A and site B were reported 0.1095±0.0007 and 0.1012±0.0006 in summer; 0.0628±0.0006 and 0.0598±0.0004 in monsoon; 0.1022±0.0008 and 0.0912±0.0005 in winter seasons respectively.

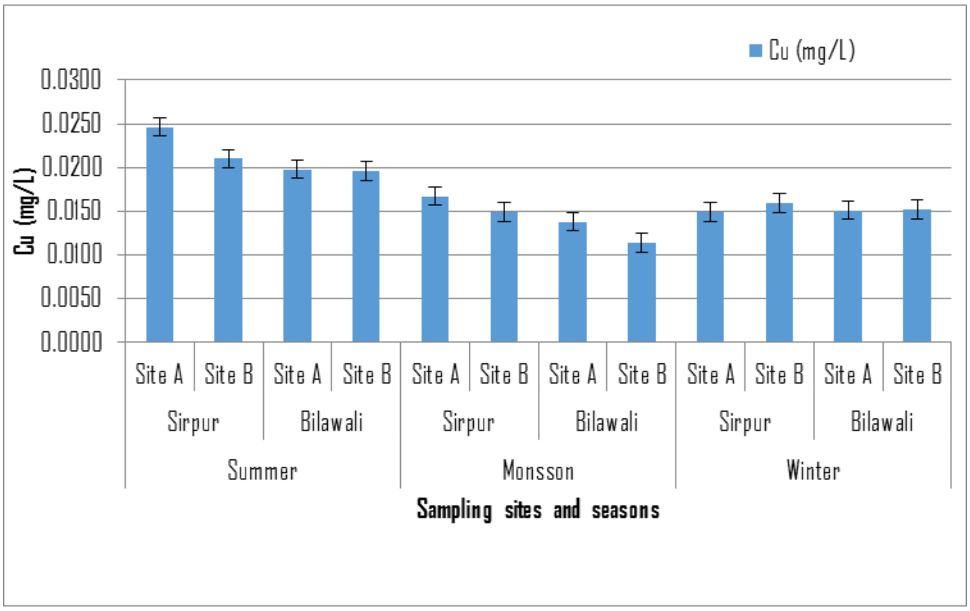
Fig. 8. Zn in water samples collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.2.1.2 Copper (Cu)

A graphical representation of copper concentration with standard error in collected water samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 2. Copper concentration with standard deviation in water samples collected from Sirpur Lake site A and site B were 0.0246±0.0004 and 0.021±0.0007 in summer; 0.0167±0.0006 and 0.0149±0.0006 in monsoon; 0.0149±0.0008 and 0.0159±0.0008 in winter seasons whereas, Bilawali Tank site A and site B were reported 0.0198±0.0006 and 0.0196±0.0005 in summer; 0.0138±0.0007 and 0.0114±0.0003 in monsoon; 0.0151±0.0009 and 0.0152±0.0002 in winter seasons respectively.

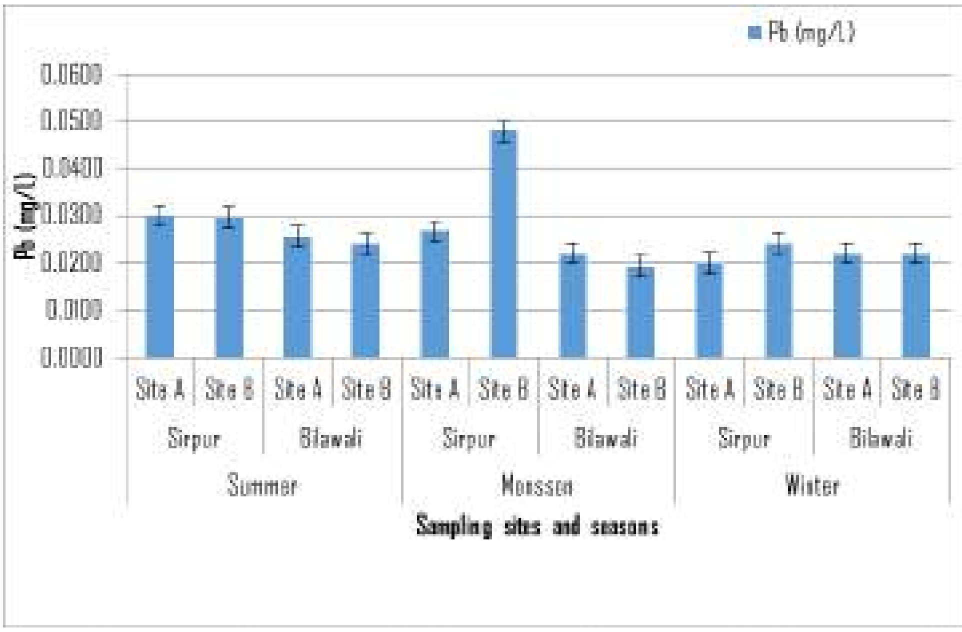
Fig. 9. Cu in water samples collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.2.1.3 Lead (Pb)

Concentration of Lead with standard error in collected water samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 2. Concentration of Lead with standard deviation in water samples collected from Sirpur Lake site A and site B were 0.0301±0.0005 and 0.0296±0.0004 in summer; 0.0268±0.0004 and 0.0480±0.0004 in monsoon; 0.0201±0.0009 and 0.0243±0.0007 in winter seasons whereas, Bilawali Tank site A and site B were reported 0.0256±0.0004 and 0.0241±0.0004in summer; 0.0221±0.0005 and 0.0194±0.0003 in monsoon; 0.0221±0.0009 and 0.0221±0.0003 in winter seasons respectively.

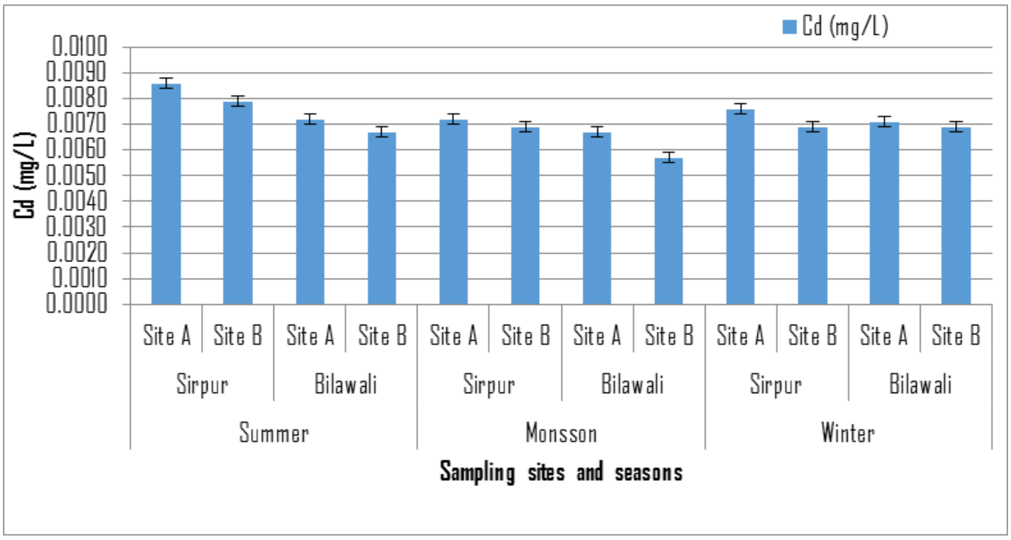
Fig. 10. Pb in water samples collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.2.1.4 Cadmium (Cd)

A graphical representation of Cadmium concentration with standard error in collected water samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 2. Cadmium concentration with standard deviation in water samples collected from Sirpur Lake site A and site B were 00.0086±0.0001 and 0.0079±0.0002 in summer; 0.0072±0.0003 and 0.0069±0.0002 in monsoon; 0.0076±0.0002 and 0.0069±0.0003 in winter seasons whereas, Bilawali Tank site A and site B were reported 0.0072±0.0002 and 0.0067±0.0001 in summer; 0.0067±0.0002 and 0.0057±0.0002 in monsoon; 0.0071±0.0003 and 0.0069±0.0001 in winter seasons respectively.

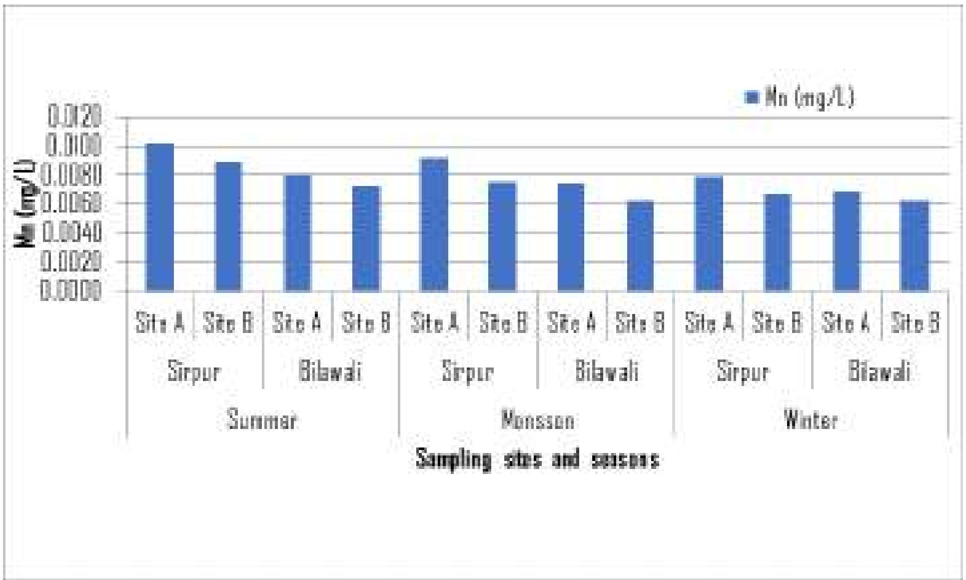
Fig. 11. Cd in water samples collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.2.1.5 Manganese

A graphical representation of Manganese concentration with standard error in collected water samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 2. Manganese concentration with standard deviation in water samples collected from Sirpur Lake site A and site B were 0.0102± 0.0002 and 0.0090± 0.0001 in summer; 0.0092± 0.0002 and 0.0076± 0.0001 in monsoon; 0.0079± 0.0001 and 0.0068± 0.0001 in winter seasons whereas, Bilawali Tank site A and site B were reported 0.0081± 0.0001 and 0.0073± 0.0001 in summer; 0.0075± 0.0001 and 0.0062± 0.0001 in monsoon; 0.0069± 0.0001 and 0.0062± 0.0001 in winter seasons respectively.

Fig. 12. Mn in water samples collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.2.2 Heavy metals in soil sediment sample:

Table 3 presents the mean with standard deviation of collected observations of heavy metals in soil sediment samples.

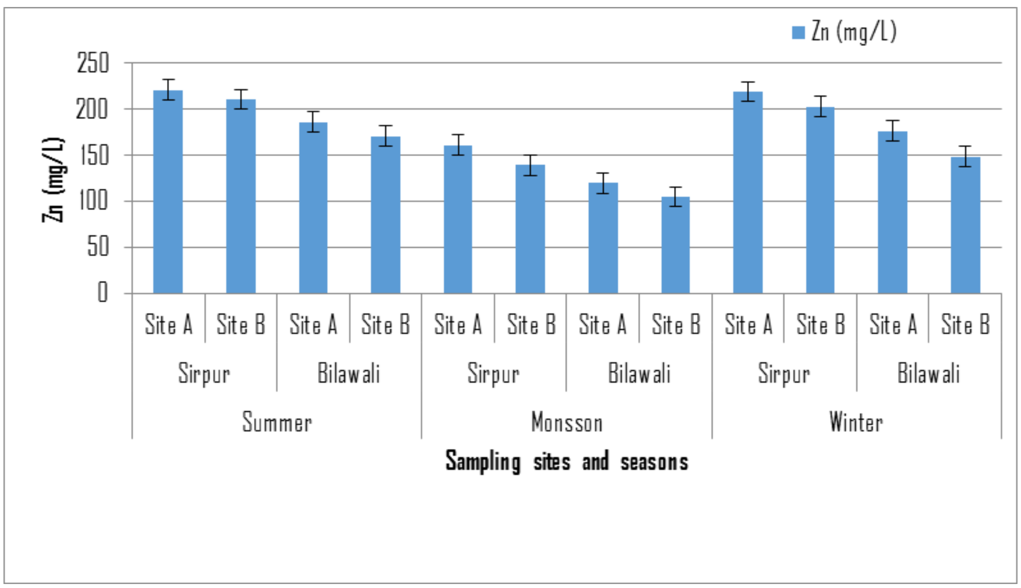
Table 3. Seasonal variations in presence of heavy metals in soil sediment samples collected from Sirpur and Bilawali Tanks of Indore.

Heavy metals in sediment soil	Summer				Monsson				Winter			
	Sirpur		Bilawali		Sirpur		Bilawali		Sirpur		Bilawali	
	Site A	Site B	Site A	Site B	Site A	Site B	Site A	Site B	Site A	Site B	Site A	Site B
Zn (mg/L)	221.36± 2.95	210.96± 2.87	186.54± 2.12	171.23± 2.46	161.51± 2.12	139.52± 2.97	120.23± 2.12	105.26± 2.41	219.26± 2.29	203.26± 3.12	176.25± 2.83	148.95± 3.98
Cu (mg/L)	69.46± 1.65	60.15± 1.41	54.26± 1.10	49.56± 0.95	51.24± 1.09	49.52± 1.42	38.65± 1.02	32.25± 1.79	59.36± 1.45	48.69± 2.01	42.15± 2.10	36.54± 2.21
Pb (mg/L)	26.51± 1.24	21.12± 0.98	19.1± 0.78	15.12± 0.93	21.02± 0.87	16.49± 0.95	14.56± 0.87	11.26± 0.42	23.12± 1.01	19.26± 1.92	17.36± 1.03	11.23± 1.16
Cd (mg/L)	4.69± 0.54	4.01± 0.41	3.96± 0.53	3.01± 0.46	3.59± 0.57	3.21± 0.39	3.01± 0.56	2.95± 0.39	4.01± 0.87	3.36± 0.82	3.47± 0.56	3.02± 0.65
Hg (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mn (mg/L)	0.029± 0.005	0.023± 0.004	0.019± 0.003	0.017± 0.003	0.021± 0.004	0.019± 0.003	0.016± 0.003	0.013± 0.004	0.023± 0.007	0.020± 0.006	0.017± 0.005	0.014± 0.004

3.2.2.1 Zinc (Zn)

Concentration of Zinc with standard error in collected soil sediment samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 3. Concentration of Zinc with standard deviation in soil sediment samples collected from Sirpur Lake site A and site B were 221.36±2.95 and 210.96±2.87 in summer; 161.51±2.12 and 139.52±2.97 in monsoon; 219.26±2.29 and 203.26±3.12 in winter seasons whereas, Bilawal tank site A and site B were reported 186.54±2.12 and 171.23±2.46 in summer; 120.23±2.12 and 105.26±2.41 in monsoon; 176.25±2.83 and 148.95±3.98 in winter seasons respectively.

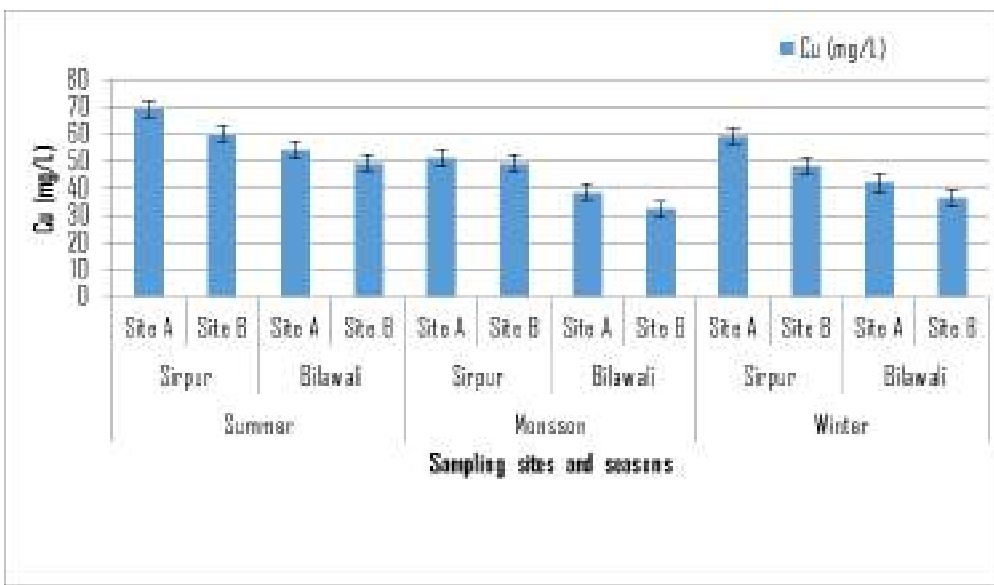
Fig. 13. Zn in soil sediment samples collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.2.2.2 Copper (Cu)

A graphical representation of copper concentration with standard error in collected soil sediment samples of Sirpur Lake and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 3. Copper concentration with standard deviation in soil sediment samples collected from Sirpur Lake site A and site B were 69.46±1.65 and 60.15±1.41 in summer; 51.24±1.09 and 49.52±1.42 in monsoon; 59.36±1.45 and 48.69±2.01 in winter seasons whereas, Bilawali Tank site A and site B were reported 54.26±1.10 and 49.56±0.95 in summer; 38.65±1.02 and 32.25±1.79 in monsoon; 42.15±2.10 and 36.54±2.21 in winter seasons respectively.

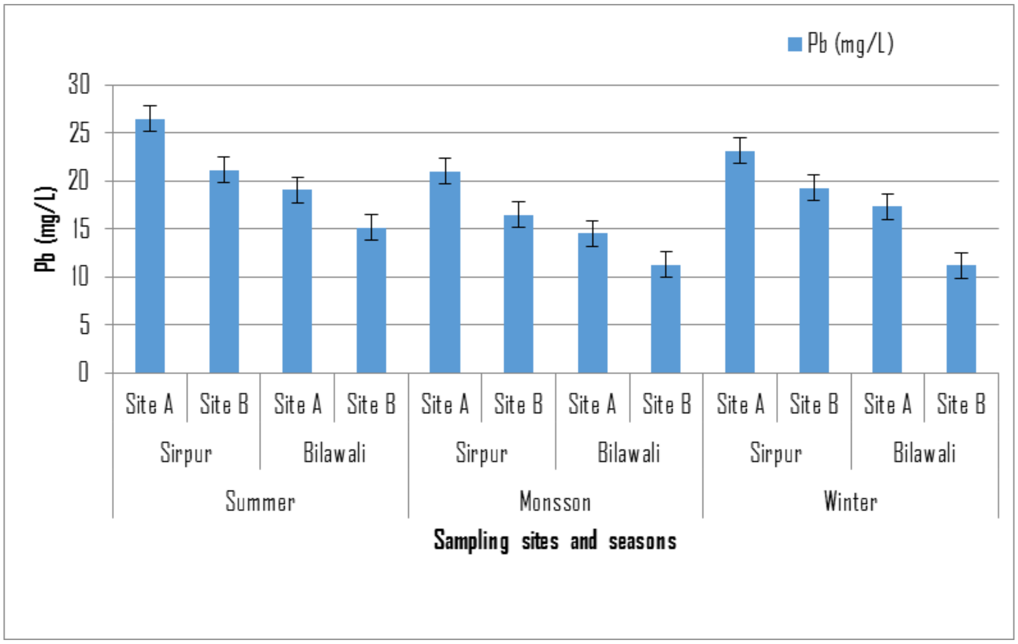
Fig. 14. Cu in soil sediment samples collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.2.2.3 Lead (Pb)

Concentration of Lead with standard error in collected soil sediment samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 3. Concentration of Lead with standard deviation in soil sediment samples collected from Sirpur Lake site A and site B were 26.51±1.24 and 21.12±0.98 in summer; 21.02±0.87 and 16.49±0.95 in monsoon; 23.12±1.01 and 19.26±1.92 in winter seasons whereas, Bilawali Tank site A and site B were reported 19.1±0.78 and 15.12±0.93 in summer; 14.56±0.87 and 11.26±0.42 in monsoon; 17.36±1.03 and 11.23±1.16 in winter seasons respectively.

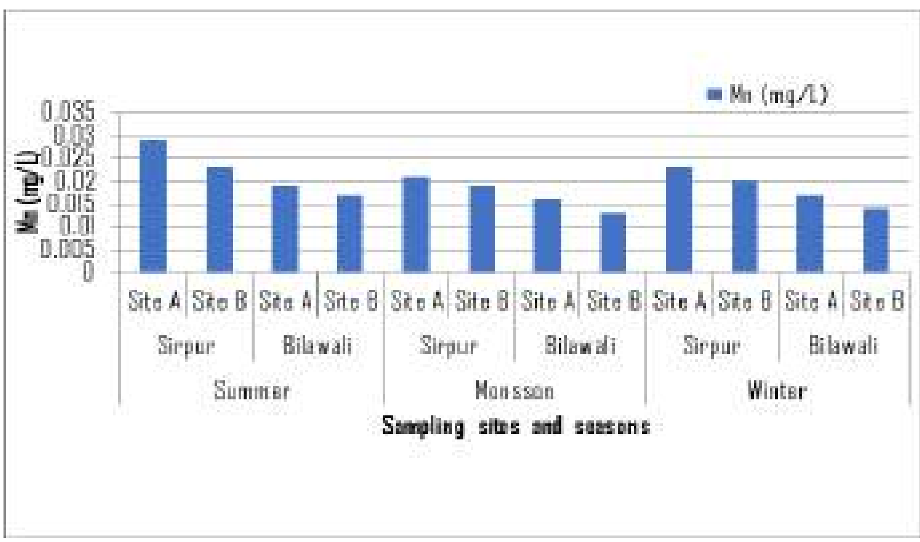
Fig. 15. Pb in soil sediment samples collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.2.2.4 Manganese (Mn)

A graphical representation of Manganese concentration with standard error in collected soil sediment samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 3. Manganese concentration with standard deviation in soil sediment samples collected from Sirpur Lake site A and site B were 0.029± 0.005 and 0.023± 0.004 in summer; 0.021± 0.004 and 0.019± 0.003 in monsoon; 0.023± 0.007 and 0.020± 0.006 in winter seasons whereas, Bilawali Tank site A and site B were reported 0.019± 0.003 and 0.017± 0.003 in summer; 0.016± 0.003 and 0.013± 0.004 in monsoon; 0.017± 0.005 and 0.014± 0.004 in winter seasons respectively

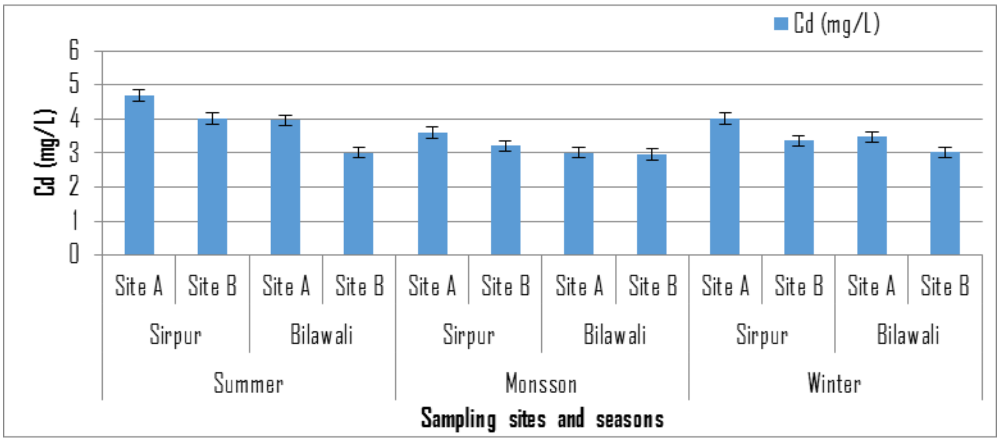
Fig. 16. Mn in soil sediment samples collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.2.2.5 Cadmium (Cd)

A graphical representation of Cadmium concentration with standard error in collected soil sediment samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 3. Cadmium concentration with standard deviation in soil sediment samples collected from Sirpur Lake site A and site B were 4.69±0.54 and 4.01±0.41 in summer; 3.59±0.57 and 3.21±0.39 in monsoon; 4.01±0.87 and 3.36±0.82 in winter seasons whereas, Bilawali Tank site A and site B were reported 3.96±0.53 and 3.01±0.46 in summer; 3.01±0.56 and 2.95±0.39 in monsoon; 3.47±0.56 and 3.02±0.65 in winter seasons respectively.

Fig. 17. Cd in soil sediment samples collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.3 Heavy metal accumulation in bivalves:

Zn, Cu, Pb, Hg, Mn and Cd heavy metals accumulation were assessed in *Lamellidens corrianus*, *Lamellidens marginali* and *Unio* sp bivalves samples collected from two different sites of Sirpur and Bilawali Tanks, Indore in summer, monsoon and winter seasons.

3.3.1 *Lamellidens corrianus*

Table 4 presents the mean with standard deviation of collected observations of heavy metals accumulation studies in *Lamellidens corrianus* samples.

Table 4. Seasonal variations of heavy metals accumulation in *Lamellidens corrianus* collected from Sirpur and Bilawali Tanks of Indore.

Parameters	Metals	Summer				Monsson				Winter			
		Sirpur		Bilawali		Sirpur		Bilawali		Sirpur		Bilawali	
		Site A	Site B	Site A	Site B	Site A	Site B	Site A	Site B	Site A	Site B	Site A	Site B
Dry weight whole body	Zn	2.22± 0.26	2.36± 0.23	2.18± 0.19	2.31± 0.21	2.15± 0.24	2.27± 0.22	2.09± 0.29	2.15± 0.23	2.22± 0.21	2.42± 0.26	2.25± 0.23	2.29± 0.24
	Cu	2.22± 0.26	2.36± 0.23	2.18± 0.19	2.31± 0.21	2.15± 0.24	2.27± 0.22	2.09± 0.29	2.15± 0.23	2.22± 0.21	2.42± 0.26	2.25± 0.23	2.29± 0.24
	Pb	2.22± 0.26	2.36± 0.23	2.18± 0.19	2.31± 0.21	2.15± 0.24	2.27± 0.22	2.09± 0.29	2.15± 0.23	2.22± 0.21	2.42± 0.26	2.25± 0.23	2.29± 0.24
	Cd	2.22± 0.26	2.36± 0.23	2.18± 0.19	2.31± 0.21	2.15± 0.24	2.27± 0.22	2.09± 0.29	2.15± 0.23	2.22± 0.21	2.42± 0.26	2.25± 0.23	2.29± 0.24
	Hg	2.22± 0.27	2.36± 0.24	2.18± 0.20	2.31± 0.22	2.15± 0.25	2.27± 0.23	2.09± 0.30	2.15± 0.24	2.22± 0.22	2.42± 0.27	2.25± 0.24	2.29± 0.25
	Mn	2.22± 0.28	2.36± 0.25	2.18± 0.21	2.31± 0.23	2.15± 0.26	2.27± 0.24	2.09± 0.31	2.15± 0.25	2.22± 0.23	2.42± 0.28	2.25± 0.25	2.29± 0.26
Metal concentration per unit body weight(µg/g)	Zn	412.56± 6.23	407.58± 6.58	389.24± 5.98	351.24± 7.10	302.26± 6.21	258.24± 6.87	234.25± 5.48	215.46± 6.23	331.28± 8.56	292.52± 7.54	284.32± 6.51	251.24± 5.41
	Cu	109.75± 2.13	101.45± 2.16	99.46± 2.84	96.25± 3.21	95.24± 2.86	85.67± 2.54	78.56± 3.10	66.51± 2.76	106.53± 3.45	93.54± 2.98	89.54± 2.56	79.86± 2.65
	Pb	122.36± 2.45	102.57± 2.42	104.57± 3.12	99.24± 2.59	99.12± 2.41	84.29± 2.10	77.19± 2.20	63.28± 2.01	109.72± 3.02	95.27± 2.75	90.53± 2.47	83.41± 2.14
	Cd	19.24± 1.12	16.51± 1.36	14.32± 1.29	12.14± 1.43	14.23± 1.34	9.84± 1.21	8.27± 1.02	6.64± 0.98	15.86± 1.11	11.2± 1.05	10.13± 1.23	8.57± 1.12
	Hg	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mn	42.3± 2.10	39.25± 1.75	30.10± 2.17	28.26± 1.95	37.54± 3.12	33.29± 3.15	26.74± 2.97	22.39± 2.85	37.6± 3.01	34.79± 3.58	29.45± 2.99	25.43± 2.86
Metal Body burden	Zn	915.86± 10.45	961.87± 11.26	848.52± 9.25	811.34± 8.57	649.84± 9.46	586.18± 8.42	489.56± 11.23	463.22± 9.35	735.42± 9.12	707.88± 11.29	639.70± 9.52	575.32± 10.23

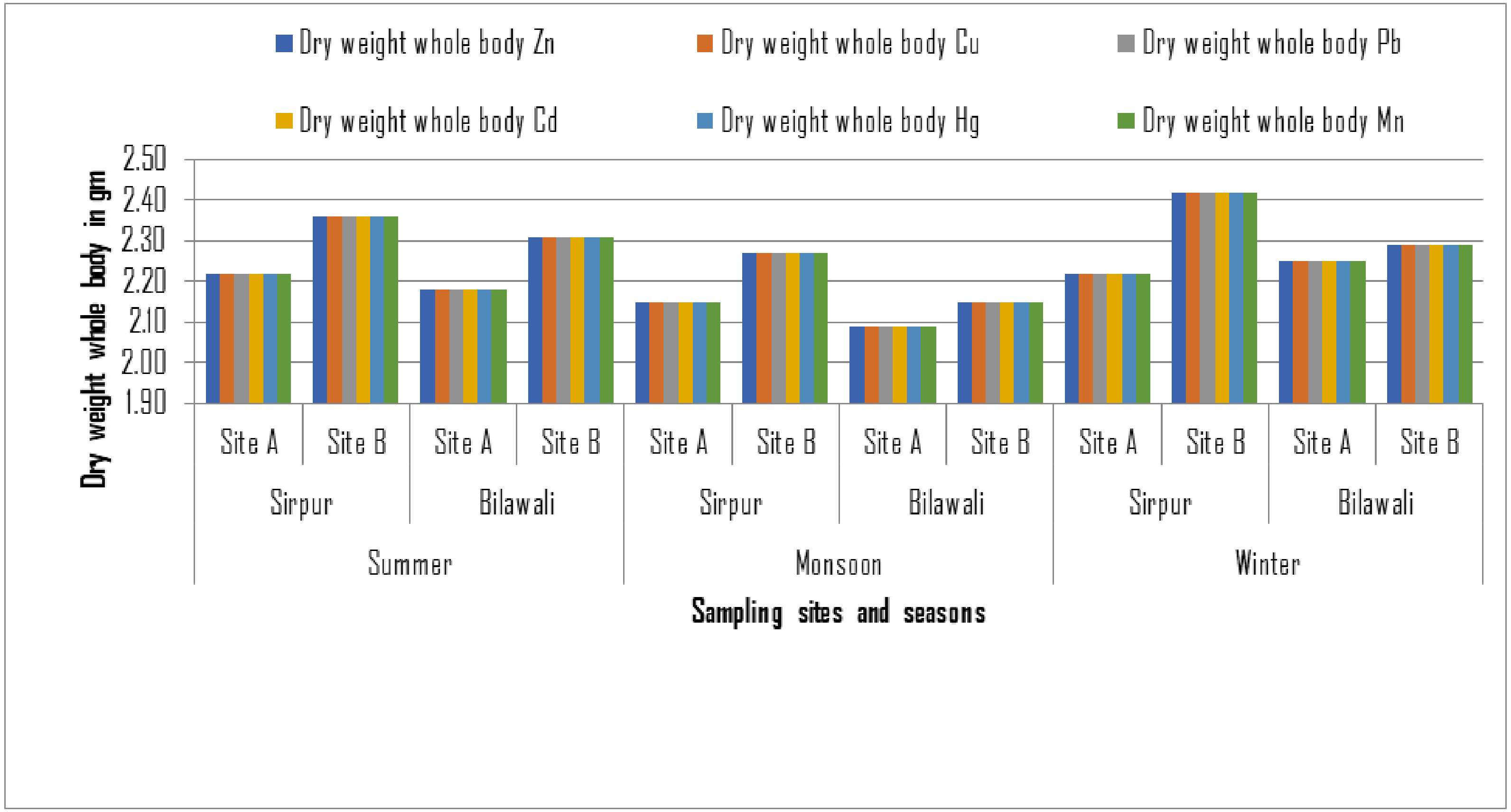
(µg/individual)													
	Cu	243.63± 5.21	239.40± 6.10	216.80± 5.65	222.32± 5.54	204.75± 4.65	194.45± 3.68	164.17± 6.54	142.98± 5.97	236.48± 8.56	226.35± 7.85	201.45± 7.12	182.86± 6.38
	Pb	271.62± 5.39	242.04± 4.95	227.94± 5.12	229.22± 4.87	213.09± 4.21	191.32± 4.03	161.31± 5.52	136.03± 4.21	243.56± 5.21	230.54± 5.96	203.67± 5.26	190.99± 6.14
	Cd	42.69± 2.56	38.94± 2.21	31.20± 1.96	28.02± 1.86	30.57± 1.96	22.32± 2.12	17.26± 1.38	14.26± 1.12	35.19± 1.36	27.08± 2.10	22.77± 2.12	19.60± 1.12
	Hg	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mn	93.90± 4.58	92.62± 5.48	65.61± 4.57	65.27± 4.38	80.70± 6.49	75.56± 7.09	55.88± 4.49	48.13± 4.28	83.46± 6.29	84.18± 6.79	66.26± 4.81	58.23± 5.71
BWAF	Zn	3125.45± 70.26	3559.65± 68.35	3554.70± 63.25	3470.75± 66.54	3369.68± 67.42	3626.97± 69.25	3730.10± 82.26	3603.01± 79.25	2783.87± 85.23	2609.45± 79.52	2781.99± 81.10	2754.82± 80.15
	Cu	4461.38± 76.24	4830.95± 71.24	5023.23± 79.21	4910.71± 76.28	5702.99± 80.54	5749.66± 77.51	5692.75± 94.57	5834.21± 82.24	7149.66± 96.24	5883.02± 81.45	5929.80± 81.22	5253.95± 86.54
	Pb	4065.12± 73.29	3465.20± 67.26	4084.77± 64.29	4117.84± 65.41	3698.51± 67.21	1756.04± 56.35	3492.76± 61.25	3261.86± 63.54	5458.71± 74.26	3920.58± 71.26	4096.38± 86.54	3774.21± 76.25
	Cd	2237.21± 51.28	2089.87± 41.02	1988.89± 32.15	1811.94± 36.52	1976.39± 41.28	1426.09± 39.85	1234.39± 41.20	1164.91± 37.62	2086.84± 42.15	1623.19± 38.57	1426.76± 39.51	1242.02± 46.58
	Hg	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mn	4147.05± 60.59	4361.11± 54.69	3716.04± 48.97	3871.23± 47.65	4080.43± 50.27	4380.26± 46.51	3565.33± 44.78	3611.29± 46.52	4759.49± 55.43	5116.17± 54.29	4268.11± 57.69	4101.61± 55.49
BSAF	Zn	1.86± 0.21	1.93± 0.19	2.09± 0.28	2.05± 0.27	1.87± 0.58	1.85± 0.12	1.95± 0.51	2.05± 0.42	1.51± 0.56	1.44± 0.46	1.61± 0.45	1.69± 0.12
	Cu	1.58± 0.12	1.69± 0.16	1.83± 0.17	1.94± 0.19	1.86± 0.29	1.73± 0.29	2.03± 0.49	2.06± 0.39	1.79± 0.33	1.92± 0.29	2.12± 0.29	2.18± 0.26
	Pb	4.62± 0.46	4.86± 0.39	5.47± 0.46	6.56± 0.42	4.72± 0.33	5.12± 0.51	5.30± 0.46	5.62± 0.49	4.75± 0.36	4.95± 0.68	5.21± 0.58	7.43± 0.35
	Cd	4.10± 0.41	4.12± 0.33	3.62± 0.41	4.03± 0.36	3.96± 0.37	3.06± 0.34	2.75± 0.32	2.25± 0.29	3.95± 0.24	3.33± 0.59	2.92± 0.21	2.84± 0.33
	Hg	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mn (x1000)	1.45± 0.04	1.70± 0.04	1.58± 0.03	1.66± 0.03	1.78± 0.04	1.75± 0.04	1.67± 0.03	1.72± 0.03	1.63± 0.04	1.73± 0.04	1.73± 0.04	1.81± 0.05

3.3.1.1 Dry weight whole body

Dry weight whole body with standard error in collected *Lamellidens corrianus* bivalve samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in figure 1. Dry weight whole

body with standard deviation in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 2.22±0.26 and 2.36±0.23 in summer; 2.15±0.24 and 2.27±0.22 in monsoon; 2.22±0.21 and 2.42±0.26 in winter seasons whereas, Bilawali Tank site A and site B were reported 2.18±0.19 and 2.31±0.21 in summer; 2.09±0.29 and 2.15±0.23 in monsoon; 2.25±0.23 and 2.29±0.24 in winter seasons respectively for all heavy metal accumulation.

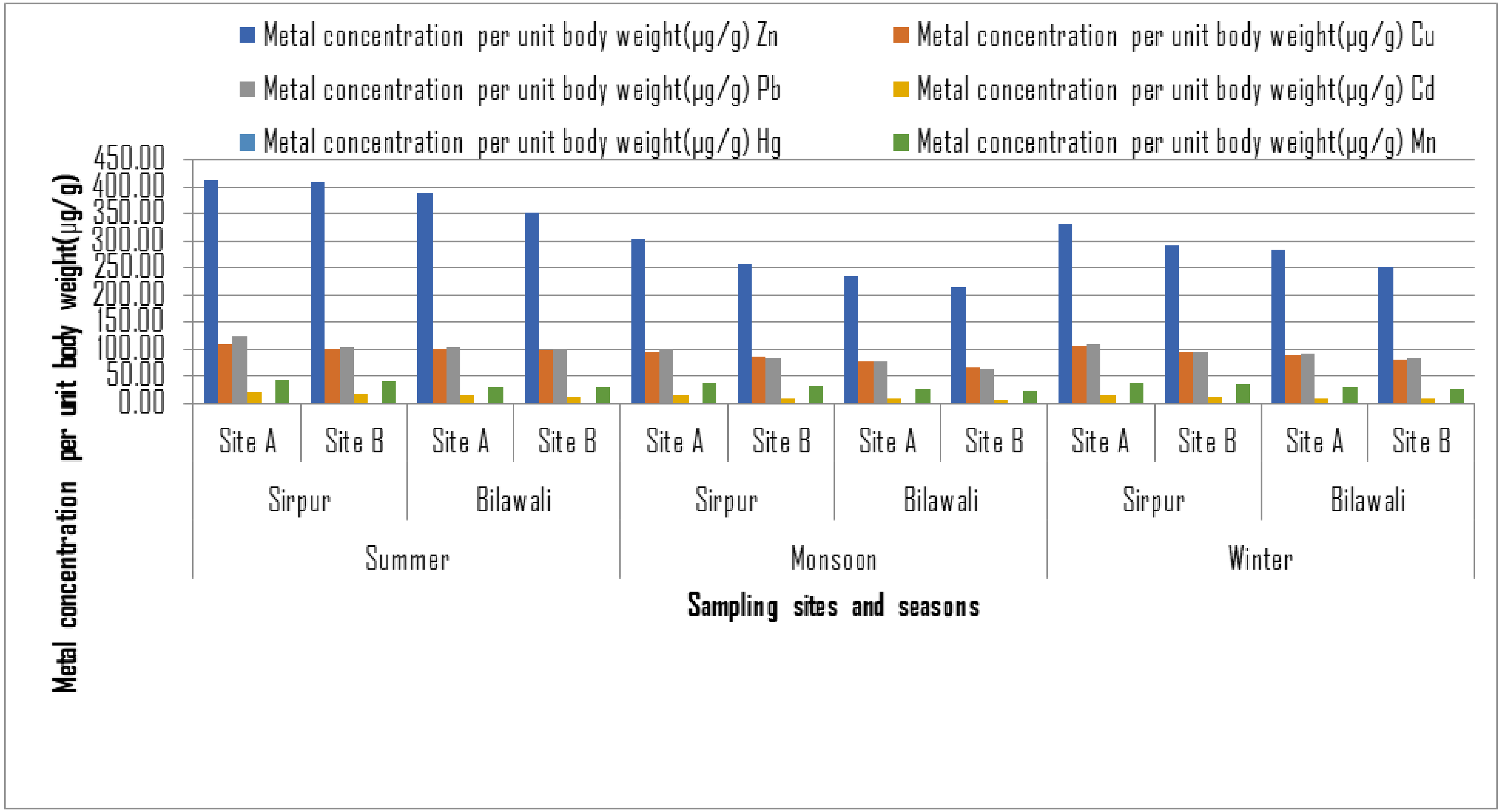
Fig. 18. Dry weight whole body of *Lamellidens corrianus* bivalves collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.3.1.2 Metal concentration per unit body weight:

Metal concentration per unit body weight with standard error in collected *Lamellidens corrianus* bivalve samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 4. Concentration of Zinc per unit body weight with standard deviation in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 412.56±6.23 and 407.58±6.58 in summer; 302.26±6.21 and 258.24±6.87 in monsoon; 331.28±8.56 and 292.52±7.54 in winter seasons whereas, Bilawali Tank site A and site B were reported 389.24±5.98 and 351.24±7.10 in summer; 234.25±5.48 and 215.46±6.23 in monsoon; 284.32±6.51 and 251.24±5.41 in winter seasons respectively. Concentration of Copper per unit body weight with standard deviation in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 109.75±2.13 and 101.45±2.16 in summer; 95.24±2.86 and 85.67±2.54 in monsoon; 106.53±3.45 and 93.54±2.98 in winter seasons whereas, Bilawali Tank site A and site B were reported 99.46±2.84 and 96.25±3.21 in summer; 78.56±3.10 and 66.51±2.76 in monsoon; 89.54±2.56 and 79.86±2.65 in winter seasons respectively. Concentration of Lead per unit body weight with standard deviation in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 122.36±2.45 and 102.57±2.42 in summer; 99.12±2.41 and 84.29±2.10 in monsoon; 109.72±3.02 and 95.27±2.75 in winter seasons whereas, Bilawali Tank site A and site B were reported 104.57±3.12 and 99.24±2.59 in summer; 77.19±2.20 and 63.28±2.01 in monsoon; 90.53±2.47 and 83.41±2.14 in winter seasons respectively. Concentration of Cadmium per unit body weight with standard deviation in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 19.24±1.12 and 16.51±1.36 in summer; 14.23±1.34 and 9.84±1.21 in monsoon; 15.86±1.11 and 11.2±1.05 in winter seasons whereas, Bilawali Tank site A and site B were reported 14.32±1.29 and 12.14±1.43 in summer; 8.27±1.02 and 6.64±0.98 in monsoon; 10.13±1.23 and 8.57±1.12 in winter seasons respectively. Concentration of Manganese per unit body weight with standard deviation in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 42.3± 2.10and 39.25± 1.75 in summer; 37.54± 3.12 and 33.29± 3.15 in monsoon; 37.6± 3.01 and 34.79± 3.58 in winter seasons whereas, Bilawali Tank site A and site B were reported 30.10± 2.17 and 28.26± 1.95 in summer; 26.74± 2.97 and 22.39± 2.85 in monsoon; 29.45± 2.99 and 25.43± 2.86 in winter seasons respectively

Fig. 19. Metal concentration per unit body weight in *Lamellidens corrianus* bivalves collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.

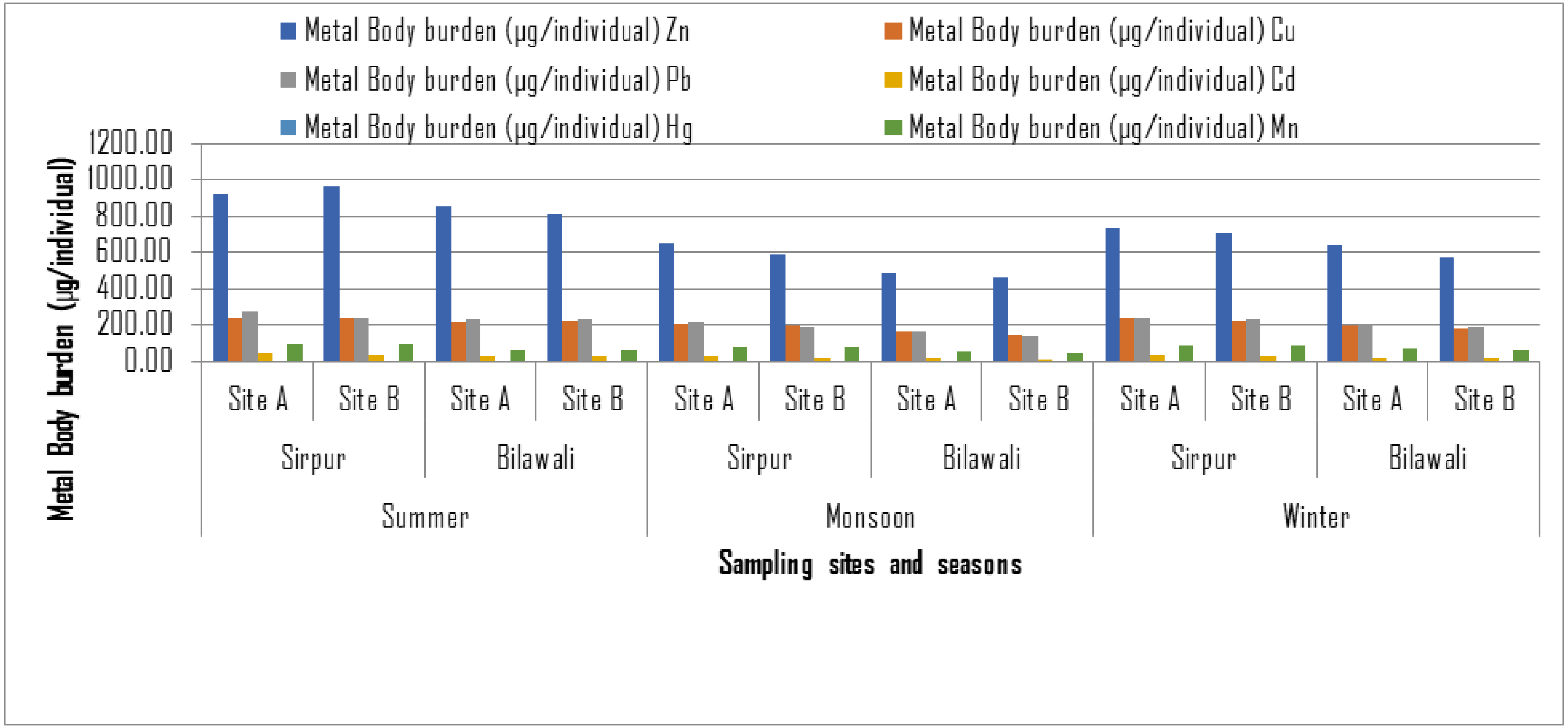


3.3.1.3 Metal Body burden (µg/individual)

Different metal body burden per individual with standard error in collected *Lamellidens corrianus* bivalve samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 4. Metal body burden of Zinc per individual with standard deviation in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 915.86±10.45 and 961.87±11.26 in summer; 649.84±9.46 and 586.18±8.42 in monsoon; 735.42±9.12 and 707.88±11.29 in winter seasons whereas, Bilawali Tank site A and site B were reported 848.52±9.25 and 811.34±8.57 in summer; 489.56±11.23 and 463.22±9.35 in monsoon; 639.70±9.52 and 575.32±10.23 in winter seasons respectively. Metal body burden of Copper per individual with standard deviation in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 243.63±5.21 and 239.40±6.10 in summer; 204.75±4.65 and 194.45±3.68 in monsoon; 236.48±8.56 and 226.35±7.85 in winter seasons whereas, Bilawali Tank site A and site B were reported 216.80±5.65 and 222.32±5.54 in summer; 164.17±6.54 and 142.98±5.97 in monsoon; 201.45±7.12 and 182.86±6.38 in winter seasons respectively. Metal body burden of Lead per individual with standard deviation in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 271.62±5.39 and 242.04±4.95 in summer; 213.09±4.21 and 191.32±4.03 in monsoon; 243.56±5.21 and

230.54±5.96 in winter seasons whereas, Bilawali Tank site A and site B were reported 227.94±5.12 and 229.22±4.87 in summer; 161.31±5.52 and 136.03±4.21 in monsoon; 203.67±5.26 and 190.99±6.14 in winter seasons respectively. Metal body burden of Manganese per individual with standard deviation in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 93.90± 4.58 and 92.62± 5.48 in summer; 80.70± 6.49 and 75.56± 7.09 in monsoon; 83.46± 6.29 and 84.18± 6.79 in winter seasons whereas, Bilawali Tank site A and site B were reported 65.61± 4.57 and 65.27± 4.38 in summer; 55.88± 4.49 and 48.13± 4.28 in monsoon; 66.26± 4.81and 58.23± 5.71 in winter seasons respectively.Metal body burden of Cadmium per individual with standard deviation in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 42.69±2.56 and 38.94±2.21 in summer; 30.57±1.96 and 22.32±2.12 in monsoon; 35.19±1.36 and 27.08±2.10 in winter seasons whereas, Bilawali Tank site A and site B were reported 31.20±1.96 and 28.02±1.86 in summer; 17.26±1.38 and 14.26±1.12 in monsoon; 22.77±2.12 and 19.60±1.12 in winter seasons respectively.

Fig. 20. Metal Body burden in *Lamellidens corrianus* bivalves collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.

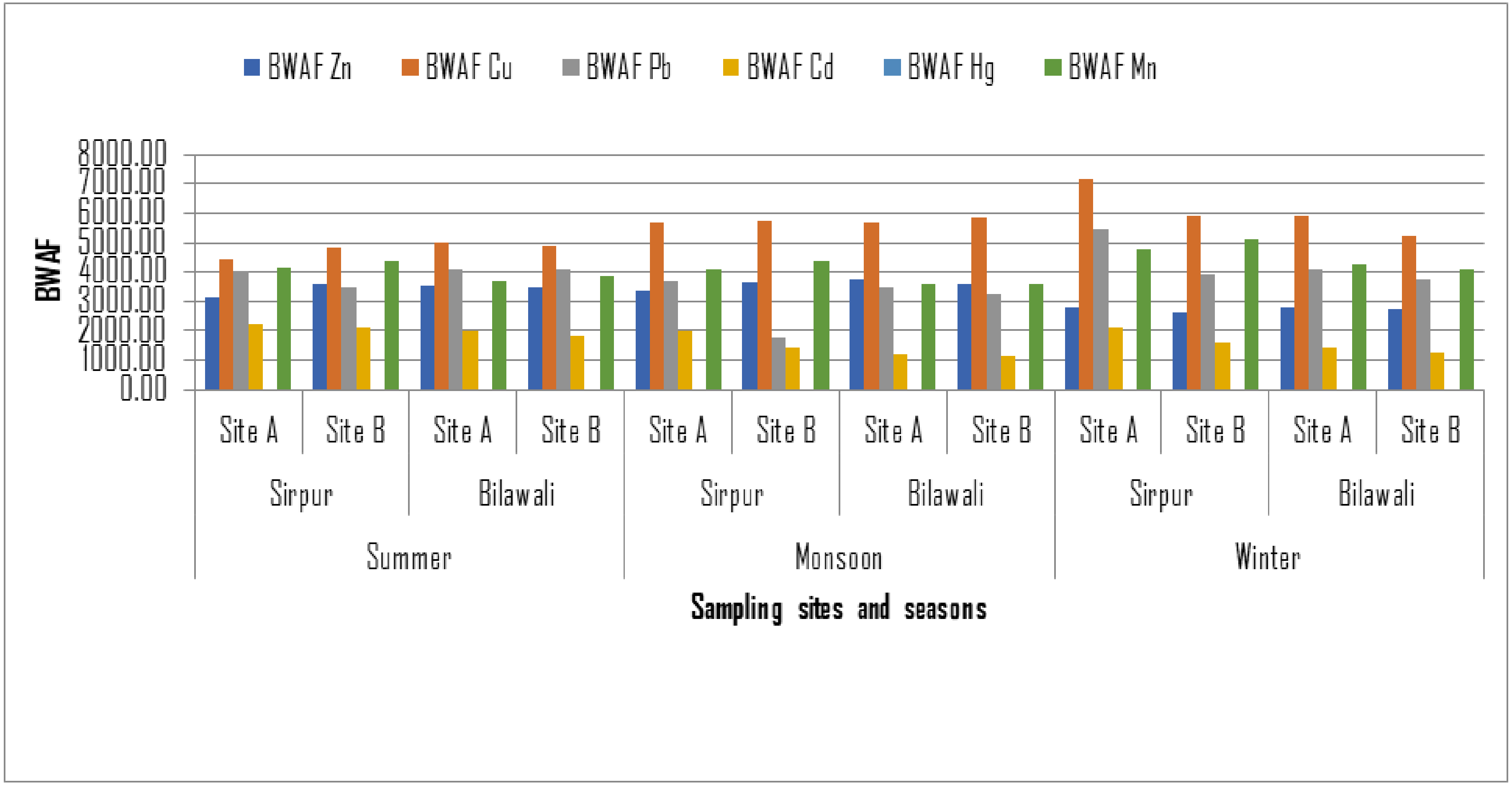


3.3.1.4 BWAF (Bio-water accumulation factor)

Bio-water accumulation factor of different metal per individual with standard error in collected *Lamellidens corrianus* bivalve samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 4. Bio-water accumulation factor of Zinc per individual with standard deviation in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 3125.45±70.26 and 3559.65±68.35 in summer; 3369.68±67.42 and 3626.97±69.25 in monsoon; 2783.87±85.23 and 2609.45±79.52 in winter seasons whereas, Bilawali Tank site A and site B were reported 3554.70±63.25 and 3470.75±66.54 in summer; 3730.10±82.26 and 3603.01±79.25 in monsoon; 2781.99±81.10 and 2754.82±80.15 in winter seasons respectively. Bio-water accumulation factor of Copper per individual with standard deviation in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 4461.38±76.24 and 4830.95±71.24 in summer; 5702.99±80.54 and 5749.66±77.51 in monsoon; 7149.66±96.24 and 5883.02±81.45 in winter seasons whereas, Bilawali Tank site A and site B were reported 5023.23±79.21 and 4910.71±76.28 in summer; 5692.75±94.57 and 5834.21±82.24 in monsoon; 5929.80±81.22 and 5253.95±86.54 in winter seasons respectively. Bio-water accumulation factor of Lead per individual with standard deviation in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 4065.12±73.29 and

3465.20±67.26 in summer; 3698.51±67.21 and 1756.04±56.35 in monsoon; 5458.71±74.26 and 3920.58±71.26 in winter seasons whereas, Bilawali Tank site A and site B were reported 4084.77±64.29 and 4117.84±65.41 in summer; 3492.76±61.25 and 3261.86±63.54 in monsoon; 4096.38±86.54 and 3774.21±76.25 in winter seasons respectively. Bio-water accumulation factor of Cadmium per individual with standard deviation in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 2237.21±51.28 and 2089.87±41.02 in summer; 1976.39±41.28 and 1426.09±39.85 in monsoon; 2086.84±42.15 and 1623.19±38.57 in winter seasons whereas, Bilawali Tank site A and site B were reported 1988.89±32.15 and 1811.94±36.52 in summer; 1234.39±41.20 and 1164.91±37.62 in monsoon; 1426.76±39.51 and 1242.02±46.58 in winter seasons respectively. Bio-water accumulation factor of Manganese per individual with standard deviation in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 4147.05± 60.59 and 4361.11± 54.69 in summer; 4080.43± 50.27 and 4380.26± 46.51 in monsoon; 4759.49± 55.43 and 5116.17± 54.29 in winter seasons whereas, Bilawali Tank site A and site B were reported 3716.04± 48.97 and 3871.23± 47.65 in summer; 3565.33± 44.78 and 3611.29± 46.52 in monsoon; 4268.11± 57.69 and 4101.61± 55.49 in winter seasons respectively

Fig. 21. BWAF in *Lamellidens corrianus* bivalves collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.

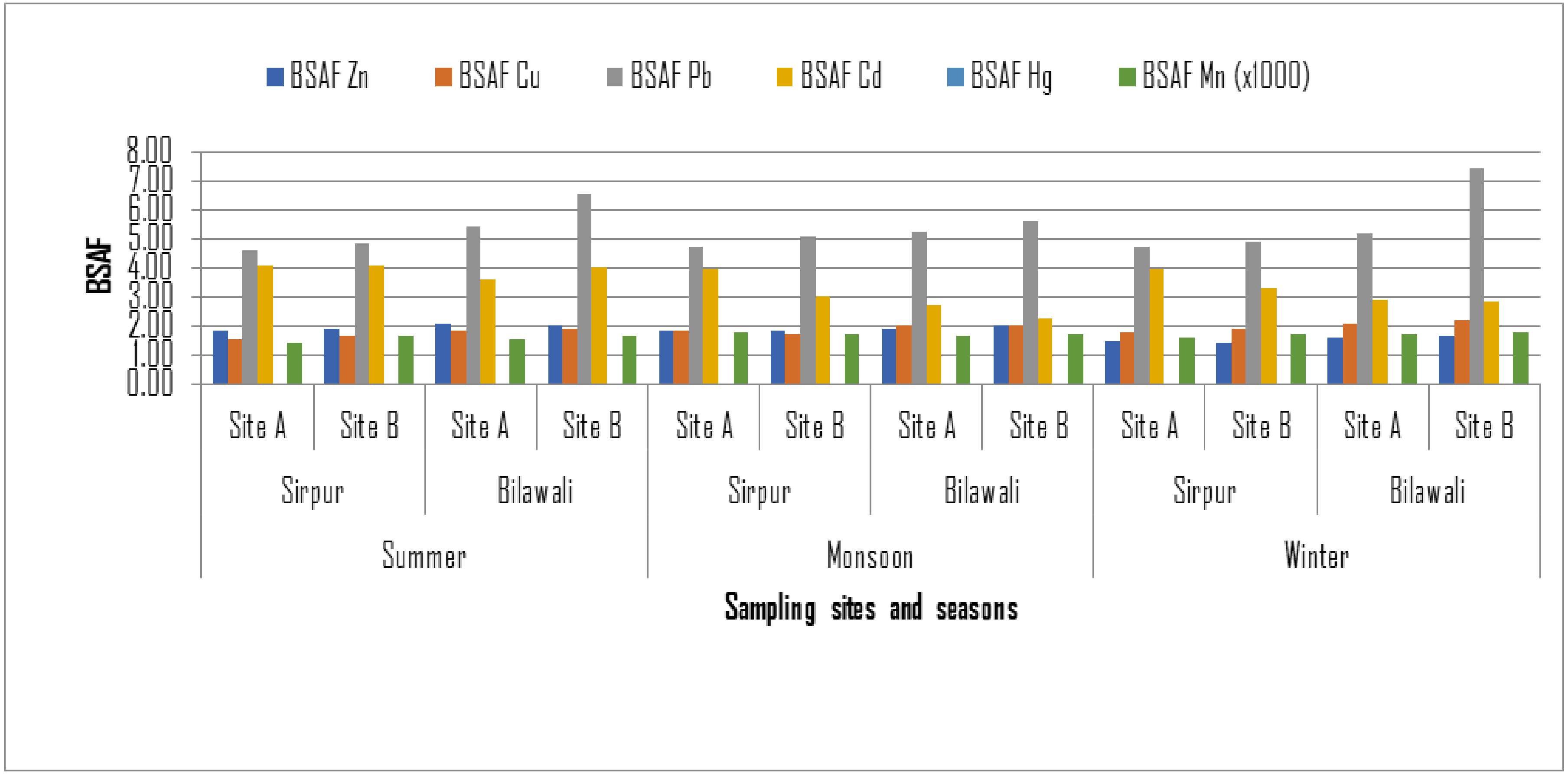


3.3.1.5 BSAF (Bio-sediment accumulation factor)

Bio- sediment accumulation factor of different metal per individual with standard error in collected *Lamellidens corrianus* bivalve samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 4. Bio- sediment accumulation factor of Zinc per individual with standard deviation in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 1.86±0.21 and 1.93±0.19 in summer; 1.87±0.58 and 1.85±0.12 in monsoon; 1.51±0.56 and 1.44±0.46 in winter seasons whereas, Bilawali Tank site A and site B were reported 2.09±0.28 and 2.05±0.27 in summer; 1.95±0.51 and 2.05±0.42 in monsoon; 1.61±0.45 and 1.69±0.12 in winter seasons respectively. Bio- sediment accumulation factor of Copper per individual with standard deviation in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 1.58±0.12 and 1.69±0.16 in summer; 1.86±0.29 and 1.73±0.29 in monsoon; 1.79±0.33 and 1.92±0.29 in winter seasons whereas, Bilawali Tank site A and site B were reported 1.83±0.17 and 1.94±0.19 in summer; 2.03±0.49 and 2.06±0.39 in monsoon; 2.12±0.29 and 2.18±0.26 in winter seasons respectively. Bio- sediment accumulation factor of Lead per individual with standard deviation in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 4.62±0.46 and 4.86±0.39 in summer; 4.72±0.33 and 5.12±0.51 in monsoon; 4.75±0.36 and 4.95±0.68 in winter seasons whereas, Bilawali

Tank site A and site B were reported 5.47±0.46 and 6.56±0.42 in summer; 5.30±0.46 and 5.62±0.49 in monsoon; 5.21±0.58 and 7.43±0.35 in winter seasons respectively. Bio- sediment accumulation factor of Cadmium per individual with standard deviation in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 4.10±0.41 and 4.12±0.33 in summer; 3.96±0.37 and 3.06±0.34 in monsoon; 3.95±0.24 and 3.33±0.59 in winter seasons whereas, Bilawali Tank site A and site B were reported 3.62±0.41 and 4.03±0.36 in summer; 2.75±0.32 and 2.25±0.29 in monsoon; 2.92±0.21 and 2.84±0.33 in winter seasons respectively. Bio- sediment accumulation factor of Manganese per individual with standard deviation in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 1.45± 0.04 and 1.70± 0.04 in summer; 1.78± 0.04 and 1.75± 0.04 in monsoon; 1.63± 0.04 and 1.73± 0.04 in winter seasons whereas, Bilawali Tank site A and site B were reported 1.58± 0.03 and 1.66± 0.03 in summer; 1.67± 0.03 and 1.72± 0.03 in monsoon; 1.73± 0.04 and 1.81± 0.05 in winter seasons respectively

Fig. 22. BSAF in *Lamellidens corrianus* bivalves collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.3.2 *Lamellidens marginali*

Table 5 presents the mean with standard deviation of collected observations of heavy metals in *Lamellidens marginali* samples.

Table 5. Seasonal variations of heavy metals accumulation in *Lamellidens marginali* collected from Sirpur and Bilawali Tanks of Indore.

Parameters	Metals	Summer				Monsson				Winter			
		Sirpur		Bilawali		Sirpur		Bilawali		Sirpur		Bilawali	
		Site A	Site B	Site A	Site B	Site A	Site B	Site A	Site B	Site A	Site B	Site A	Site B
Dry weight whole body	Zn	2.32± 0.29	2.29± 0.26	2.18± 0.19	2.21± 0.22	2.84± 0.32	2.69± 0.25	2.54± 0.22	2.28± 0.31	2.17± 0.27	2.32± 0.15	2.19± 0.18	2.18± 0.19
	Cu	2.32± 0.29	2.29± 0.26	2.18± 0.19	2.21± 0.22	2.84± 0.32	2.69± 0.25	2.54± 0.22	2.28± 0.31	2.17± 0.27	2.32± 0.15	2.19± 0.18	2.18± 0.19
	Pb	2.32± 0.29	2.29± 0.26	2.18± 0.19	2.21± 0.22	2.84± 0.32	2.69± 0.25	2.54± 0.22	2.28± 0.31	2.17± 0.27	2.32± 0.15	2.19± 0.18	2.18± 0.19
	Cd	2.32± 0.29	2.29± 0.26	2.18± 0.19	2.21± 0.22	2.84± 0.32	2.69± 0.25	2.54± 0.22	2.28± 0.31	2.17± 0.27	2.32± 0.15	2.19± 0.18	2.18± 0.19
	Hg	2.32± 0.30	2.29± 0.27	2.18± 0.20	2.21± 0.23	2.84± 0.33	2.69± 0.26	2.54± 0.23	2.28± 0.32	2.17± 0.28	2.32± 0.16	2.19± 0.19	2.18± 0.20
	Mn	2.32± 0.31	2.29± 0.28	2.18± 0.21	2.21± 0.24	2.84± 0.34	2.69± 0.27	2.54± 0.24	2.28± 0.33	2.17± 0.29	2.32± 0.17	2.19± 0.20	2.18± 0.21
Metal concentration per unit body weight(µg/g)	Zn	429.56± 6.23	411.16± 7.12	384.49± 6.21	361.76± 5.12	301.45± 7.26	297.84± 6.20	239.56± 4.85	246.49± 4.12	334.19± 5.69	302.18± 6.87	289.68± 5.42	271.59± 4.56
	Cu	128.76± 2.36	113.24± 2.17	107.51± 1.96	97.58± 4.12	97.28± 3.65	88.10± 3.12	81.47± 2.64	73.24± 3.02	116.24± 3.57	99.75± 2.45	96.85± 2.61	86.41± 2.43
	Pb	109.24± 1.98	106.46± 2.10	97.43± 1.64	96.76± 2.33	93.41± 2.54	79.48± 2.25	76.58± 1.95	63.15± 1.86	107.58± 3.12	95.78± 3.72	84.27± 2.84	81.26± 2.56
	Cd	24.23± 0.59	16.10± 0.23	16.24± 0.52	13.18± 0.39	13.57± 0.52	9.68± 0.32	10.12± 0.28	7.34± 0.19	17.49± 0.12	12.57± 0.34	10.29± 0.29	9.27± 0.19
	Hg	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mn	40.21± 5.20	36.52± 4.59	34.57± 4.21	32.12± 4.29	33.46± 4.59	29.41± 4.99	30.29± 5.10	26.18± 3.29	37.59± 4.27	34.27± 4.51	28.61± 4.53	24.73± 4.59
Metal Body burden (µg/individual)	Zn	996.73± 23.69	941.71± 26.51	838.34± 32.14	799.64± 30.15	856.27± 28.21	801.34± 25.21	608.6324± 18.55	562.15± 16.45	725.34± 20.19	701.21± 22.42	634.55± 19.87	592.22± 20.19

	Cu	298.87± 12.28	259.47± 13.24	234.52± 11.28	215.80± 12.69	276.42± 10.20	237.14± 9.23	207.08± 8.24	167.14± 6.48	252.39± 14.29	231.57± 11.75	212.25± 10.23	188.52± 8.65
	Pb	253.59± 11.14	243.94± 12.15	212.55± 11.43	213.99± 10.42	265.44± 9.10	213.95± 8.56	194.66± 9.23	144.13± 4.23	233.60± 7.31	222.36± 4.52	184.70± 3.85	177.30± 3.02
	Cd	56.36± 5.54	37.02± 4.65	35.55± 4.53	29.28± 2.15	38.69± 2.59	26.19± 3.10	25.85± 2.18	16.88± 1.96	38.10± 2.43	29.31± 1.96	22.68± 1.57	20.36± 1.28
	Hg	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mn	93.30± 9.52	83.64± 9.12	75.37± 8.42	70.99± 8.14	95.04± 7.59	79.12± 7.34	76.95± 6.24	59.70± 5.54	81.58± 7.21	79.52± 5.49	62.67± 6.28	53.92± 5.47
BWAF	Zn	3254.24± 56.24	3590.92± 61.92	3511.32± 63.25	3574.70± 55.42	3360.65± 52.14	4183.15± 56.24	3814.65± 49.25	4121.91± 42.56	2808.32± 53.47	2695.63± 52.51	2834.44± 51.24	2977.96± 55.26
	Cu	5234.15± 64.12	5392.38± 69.54	5429.80± 61.25	4978.57± 59.23	5825.12± 62.14	5912.75± 65.14	5903.62± 52.67	6424.56± 68.24	7801.34± 47.91	6273.58± 64.23	6413.91± 41.28	5684.87± 43.10
	Pb	3629.23± 51.13	3596.62± 55.43	3805.86± 44.23	4014.94± 39.57	3485.48± 51.12	1655.83± 23.58	3465.16± 31.50	3255.15± 30.58	5352.24± 38.46	3941.56± 34.29	3813.12± 52.10	3676.92± 49.82
	Cd	2817.44± 34.29	2037.97± 38.23	2255.56± 34.16	1967.16± 29.21	1884.72± 22.82	1402.90± 20.15	1510.45± 18.25	1287.72± 14.25	2301.32± 19.75	1821.74± 14.20	1449.30± 12.29	1343.48± 11.29
	Hg	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mn	3942.15± 68.59	4057.77± 71.42	4267.90± 75.43	4400.00± 80.27	3636.95± 66.51	3869.73± 64.28	4038.66± 48.59	4222.58± 61.27	4758.22± 63.19	5039.70± 52.19	4146.37± 50.47	3988.70± 54.28
BSAF	Zn	1.94± 0.12	1.95± 0.19	2.06± 0.23	2.11± 0.12	1.87± 0.19	2.13± 0.24	1.99± 0.15	2.34± 0.16	1.52± 0.12	1.49± 0.17	1.64± 0.17	1.82± 0.13
	Cu	1.85± 0.10	1.88± 0.16	1.98± 0.21	1.97± 0.22	1.90± 0.20	1.78± 0.11	2.10± 0.27	2.27± 0.21	1.96± 0.15	2.05± 0.11	2.29± 0.13	2.36± 0.16
	Pb	4.12± 2.14	5.04± 2.22	5.10± 2.10	6.40± 0.24	4.44± 0.43	4.82± 0.23	5.26± 0.29	5.60± 0.36	4.65± 0.29	4.97± 0.22	4.85± 0.13	7.23± 0.18
	Cd	5.17± 3.45	4.01± 1.06	4.10± 1.95	4.38± 0.21	3.78± 0.32	3.01± 0.19	3.36± 0.10	2.49± 0.21	4.36± 0.18	3.74± 0.020	2.96± 0.16	3.07± 0.27
	Hg	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mn (x1000)	1.38± 0.03	1.58± 0.04	1.81± 0.04	1.88± 0.05	1.59± 0.03	1.54± 0.03	1.89± 0.04	2.01± 0.05	1.63± 0.04	1.71± 0.04	1.68± 0.03	1.76± 0.04

3.3.2.1 Dry weight whole body

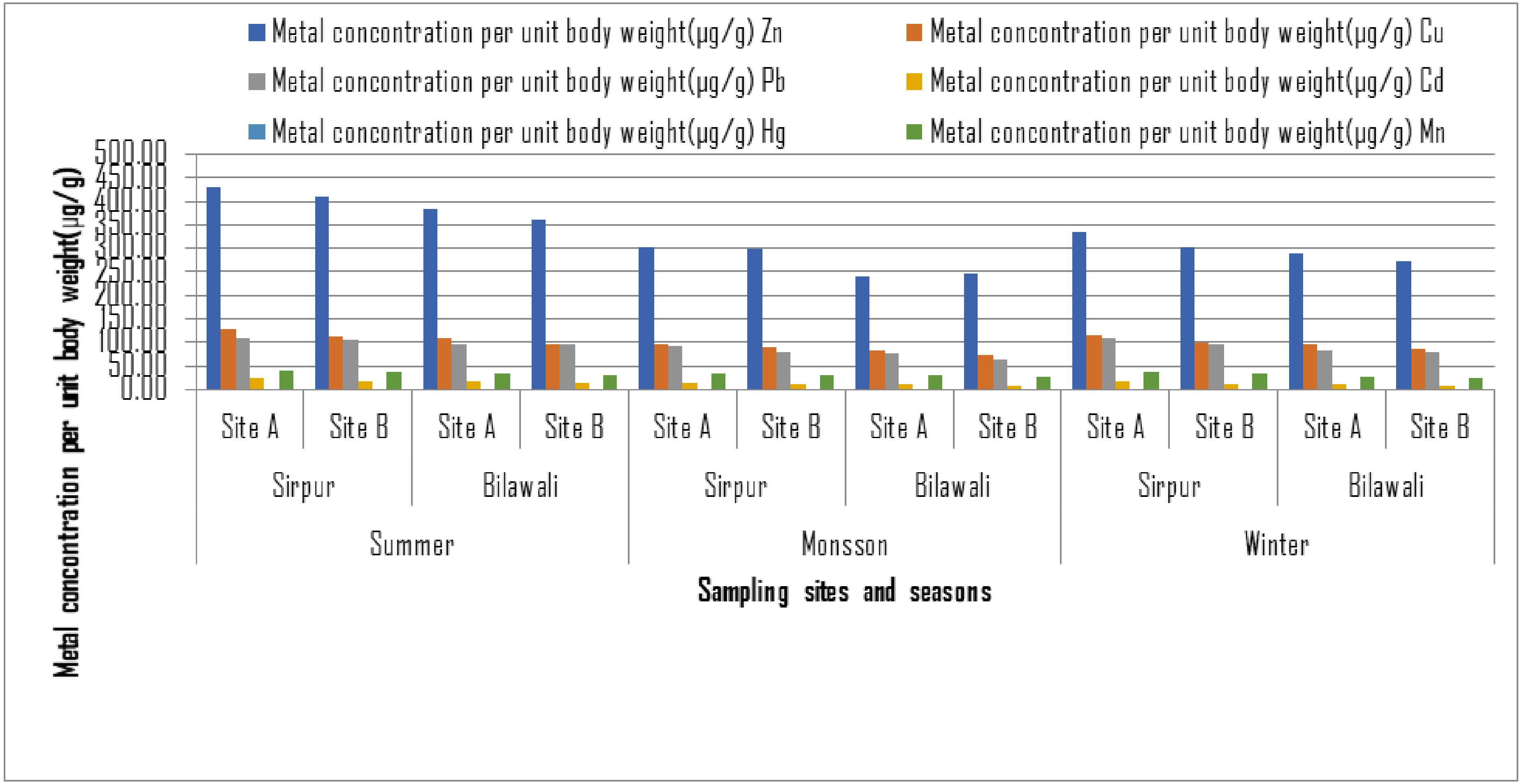
Dry weight whole body with standard error in collected *Lamellidens marginali* bivalve samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 5. Dry weight whole body with standard deviation in *Lamellidens marginali* bivalve samples collected from Sirpur Lake site A and site B were 2.32±0.29 and 2.29±0.26 in summer; 2.84±0.32 and 2.69±0.25 in monsoon; 2.17±0.27 and 2.32±0.15 in winter seasons whereas, Bilawali Tank site A and site B were reported 2.18±0.19 and 2.21±0.22 in summer; 2.54±0.22 and 2.28±0.31 in monsoon2.19±0.18 and 2.18±0.19 in winter seasons respectively for all heavy metal accumulation.

Fig. 23. Dry weight whole body of *Lamellidens marginali* bivalves collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.

3.3.2.2 Metal concentration per unit body weight (μg/g)

Metal concentration per unit body weight with standard error in collected *Lamellidens marginali* bivalve samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 5. Concentration of Zinc per unit body weight with standard deviation in *Lamellidens marginali* bivalve samples collected from Sirpur Lake site A and site B were 429.56±6.23 and 411.16±7.12 in summer; 301.45±7.26 and 297.84±6.20 in monsoon; 334.19±5.69 and 302.18±6.87 in winter seasons whereas, Bilawali Tank site A and site B were reported 384.49±6.21 and 361.76±5.12 in summer; 239.56±4.85 and 246.49±4.12 in monsoon; 289.68±5.42 and 271.59±4.56 in winter seasons respectively. Concentration of Copper per unit body weight with standard deviation in *Lamellidens marginali* bivalve samples collected from Sirpur Lake site A and site B were 128.76±2.36 and 113.24±2.17 in summer; 97.28±3.65 and 88.10±3.12 in monsoon; 116.24±3.57 and 99.75±2.45 in winter seasons whereas, Bilawali Tank site A and site B were reported 107.51±1.96 and 97.58±4.12 in summer; 81.47±2.64 and 73.24±3.02 in monsoon; 96.85±2.61 and 86.41±2.43 in winter seasons respectively. Concentration of Lead per unit body weight with standard deviation in *Lamellidens marginali* bivalve samples collected from Sirpur Lake site A and site B were 109.24±1.98 and 106.46±2.10 in summer; 93.41±2.54 and 79.48±2.25 in monsoon; 107.58±3.12 and 95.78±3.72 in winter seasons whereas, Bilawali Tank site A and site B were reported 97.43±1.64 and 96.76±2.33 in summer; 76.58±1.95 and 63.15±1.86 in monsoon; 84.27±2.84 and 81.26±2.56 in winter seasons respectively. Concentration of Cadmium per unit body weight with standard deviation in *Lamellidens marginali* bivalve samples collected from Sirpur Lake site A and site B were 24.23±0.59 and 16.10±0.23 in summer; 13.57±0.52 and 9.68±0.32 in monsoon; 17.49±0.12 and 12.57±0.34 in winter seasons whereas, Bilawali Tank site A and site B were reported 16.24±0.52 and 13.18±0.39 in summer; 10.12±0.28 and 7.34±0.19 in monsoon; 10.29±0.29 and 9.27±0.19 in winter seasons respectively. Concentration of Manganese per unit body weight with standard deviation in *Lamellidens marginali* bivalve samples collected from Sirpur Lake site A and site B were 40.21± 5.20 and 36.52± 4.59 in summer; 33.46± 4.59 and 29.41± 4.99 in monsoon; 37.59± 4.27 and 34.27± 4.51 in winter seasons whereas, Bilawali Tank site A and site B were reported 34.57± 4.21 and 32.12± 4.29 in summer; 30.29± 5.10 and 26.18± 3.29 in monsoon; 28.61± 4.53 and 24.73± 4.59 in winter seasons respectively.

Fig. 24. Metal concentration per unit body weight in *Lamellidens marginali* bivalves collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.

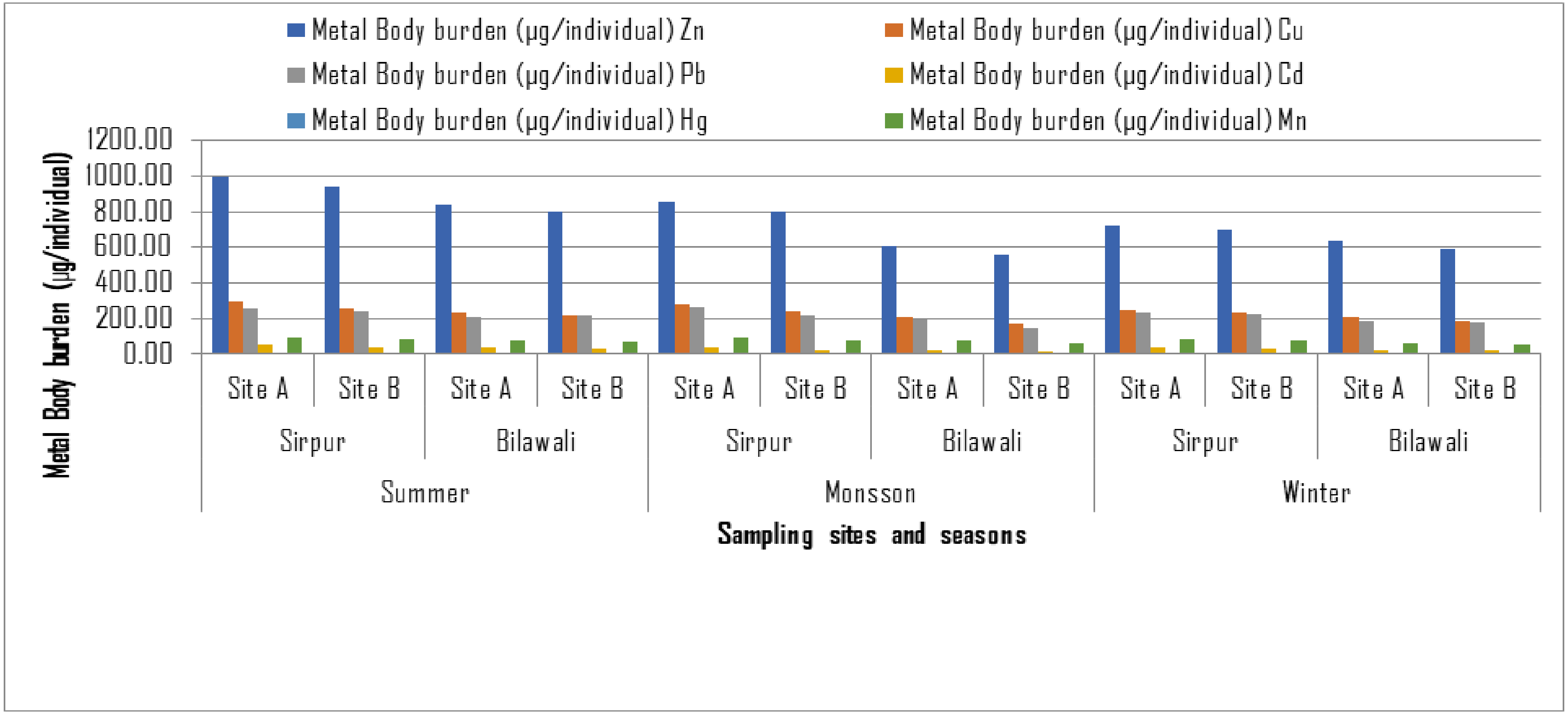


3.3.2.3 Metal Body burden (µg/individual)

Different metal body burden per individual with standard error in collected *Lamellidens marginali* bivalve samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 5. Metal body burden of Zinc per individual with standard deviation in *Lamellidens marginali* bivalve samples collected from Sirpur Lake site A and site B were 996.73±23.69 and 941.71±26.51 in summer; 856.27±28.21 and 801.34±25.21 in monsoon; 725.34±20.19 and 701.21±22.42 in winter seasons whereas, Bilawali Tank site A and site B were reported 838.34±32.14 and 799.64±30.15 in summer; 608.6324±18.55 and 562.15±16.45 in monsoon; 634.55±19.87 and 592.22±20.19 in winter seasons respectively. Metal body burden of Copper per individual with standard deviation in *Lamellidens marginali* bivalve samples collected from Sirpur Lake site A and site B were 298.87±12.28 and 259.47±13.24 in summer; 276.42±10.20 and 237.14±9.23 in monsoon; 252.39±14.29 and 231.57±11.75 in winter seasons whereas, Bilawali Tank site A and site B were reported 234.52±11.28 and 215.80±12.69 in summer; 207.08±8.24 and 167.14±6.48 in monsoon; 212.25±10.23 and 188.52±8.65 in winter seasons respectively. Metal body burden of Lead per individual with standard deviation in *Lamellidens marginali* bivalve samples collected from Sirpur Lake site A and site B were 253.59±11.14 and 243.94±12.15 in summer; 265.44±9.10 and 213.95±8.56 in monsoon;

233.60±7.31 and 222.36±4.52 in winter seasons whereas, Bilawali Tank site A and site B were reported 212.55±11.43 and 213.99±10.42 in summer; 194.66±9.23 and 144.13±4.23 in monsoon; 184.70±3.85 and 177.30±3.02 in winter seasons respectively. Metal body burden of Cadmium per individual with standard deviation in *Lamellidens marginali* bivalve samples collected from Sirpur Lake site A and site B were 56.36±5.54 and 37.02±4.65 in summer; 38.69±2.59 and 26.19±3.10 in monsoon; 38.10±2.43 and 29.31±1.96 in winter seasons whereas, Bilawali Tank site A and site B were reported 35.55±4.53 and 29.28±2.15 in summer; 25.85±2.18 and 16.88±1.96 in monsoon; 22.68±1.57 and 20.36±1.28 in winter seasons respectively. Metal body burden of Manganese per individual with standard deviation in *Lamellidens marginali* bivalve samples collected from Sirpur Lake site A and site B were 93.30± 9.52 and 83.64± 9.12 in summer; 95.04± 7.59 and 79.12± 7.34 in monsoon; 95.04± 7.59 and 76.95± 6.24 in winter seasons whereas, Bilawali Tank site A and site B were reported 75.37± 8.42 and 70.99± 8.14 in summer; 76.95± 6.24 and 59.70± 5.54in monsoon; 62.67± 6.28 and 53.92± 5.47 in winter seasons respectively.

Fig. 25. Metal Body burden in *Lamellidens marginali* bivalves collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.

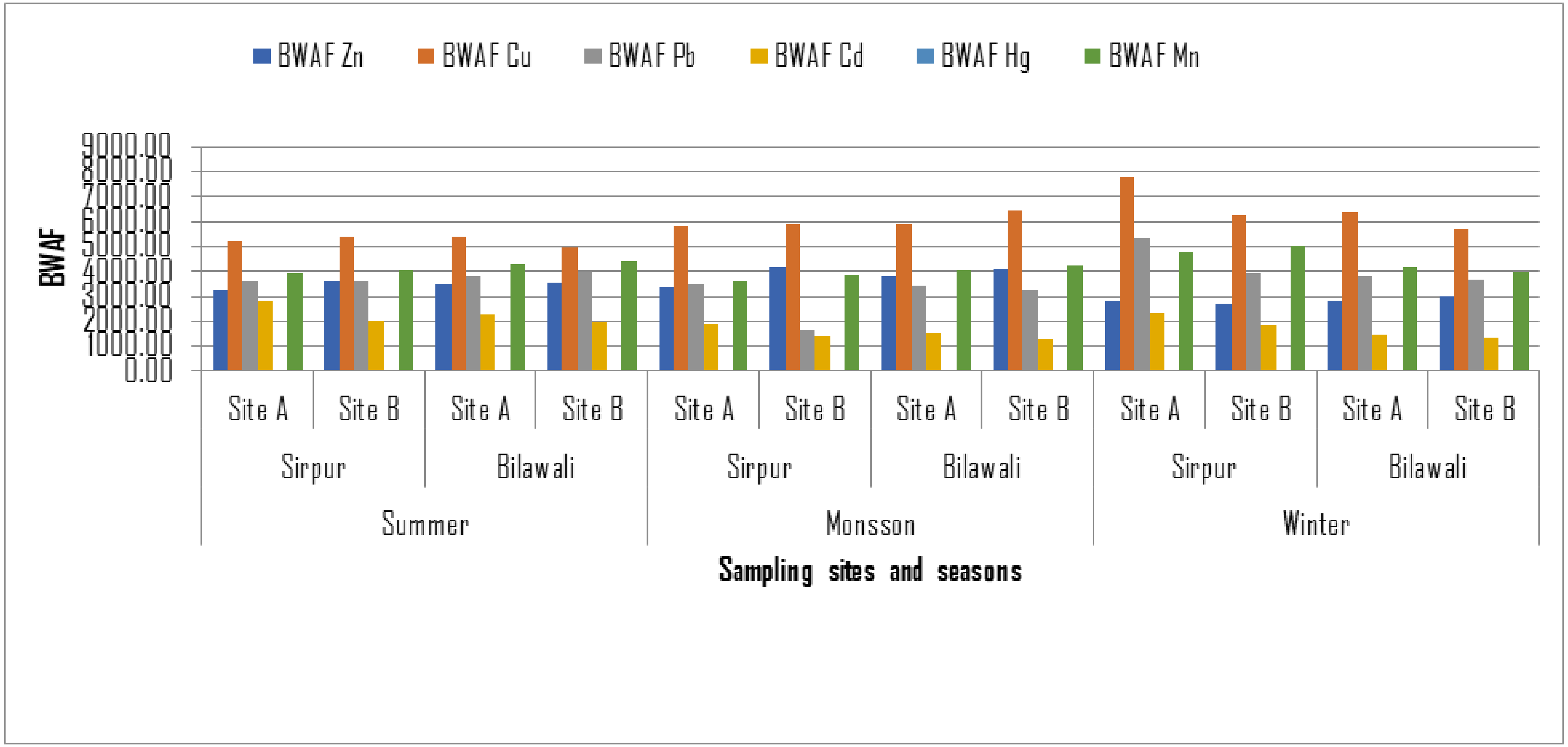


3.3.2.4 BWAF (Bio-water accumulation factor)

Bio-water accumulation factor of different metal per individual with standard error in collected *Lamellidens marginali* bivalve samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 5. Bio-water accumulation factor of Zinc per individual with standard deviation in *Lamellidens marginali* bivalve samples collected from Sirpur Lake site A and site B were 3254.24±56.24 and 3590.92±61.92 in summer; 3360.65±52.14 and 4183.15±56.24 in monsoon; 2808.32±53.47 and 2695.63±52.51 in winter seasons whereas, Bilawali Tank site A and site B were reported 3511.32±63.25 and 3574.70±55.42 in summer; 3814.65±49.25 and 4121.91±42.56 in monsoon; 2834.44±51.24 and 2977.96±55.26 in winter seasons respectively. Bio-water accumulation factor of Copper per individual with standard deviation in *Lamellidens marginali* bivalve samples collected from Sirpur Lake site A and site B were 5234.15±64.12 and 5392.38±69.54 in summer; 5825.12±62.14 and 5912.75±65.14 in monsoon; 7801.34±47.91 and 6273.58±64.23 in winter seasons whereas, Bilawali Tank site A and site B were reported 5429.80±61.25 and 4978.57±59.23 in summer; 5903.62±52.67 and 6424.56±68.24 in monsoon; 6413.91±41.28 and 5684.87±43.10 in winter seasons respectively. Bio-water accumulation factor of Lead per individual with standard deviation in *Lamellidens marginali* bivalve samples collected from Sirpur Lake site A and site B were 3629.23±51.13 and

3596.62±55.43 in summer; 3485.48±51.12 and 1655.83±23.58 in monsoon; 5352.24±38.46 and 3941.56±34.29 in winter seasons whereas, Bilawali Tank site A and site B were reported 3805.86±44.23 and 4014.94±39.57 in summer; 3465.16±31.50 and 3255.15±30.58 in monsoon; 3813.12±52.10 and 3676.92±49.82 in winter seasons respectively. Bio-water accumulation factor of Cadmium per individual with standard deviation in *Lamellidens marginali* bivalve samples collected from Sirpur Lake site A and site B were 2817.44±34.29 and 2037.97±38.23 in summer; 1884.72±22.82 and 1402.90±20.15 in monsoon; 2301.32±19.75 and 1821.74±14.20 in winter seasons whereas, Bilawali Tank site A and site B were reported 2255.56±34.16 and 1967.16±29.21 in summer; 1510.45±18.25 and 1287.72±14.25 in monsoon; 1449.30±12.29 and 1343.48±11.29 in winter seasons respectively. Bio-water accumulation factor of Manganese per individual with standard deviation in *Lamellidens marginal*i bivalve samples collected from Sirpur Lake site A and site B were 3942.15± 68.59 and 4057.77± 71.42 in summer; 3636.95± 66.51 and 3869.73± 64.28 in monsoon; 4758.22± 63.19 and 5039.70± 52.19 in winter seasons whereas, Bilawali Tank site A and site B were reported 4267.90± 75.43 .16 and 4400.00± 80.27 in summer; 4038.66± 48.59 and 4222.58± 61.27 in monsoon; 4146.37± 50.47 and 3988.70± 54.28in winter seasons respectively

Fig. 26. BWAF in *Lamellidens marginali* bivalves collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.

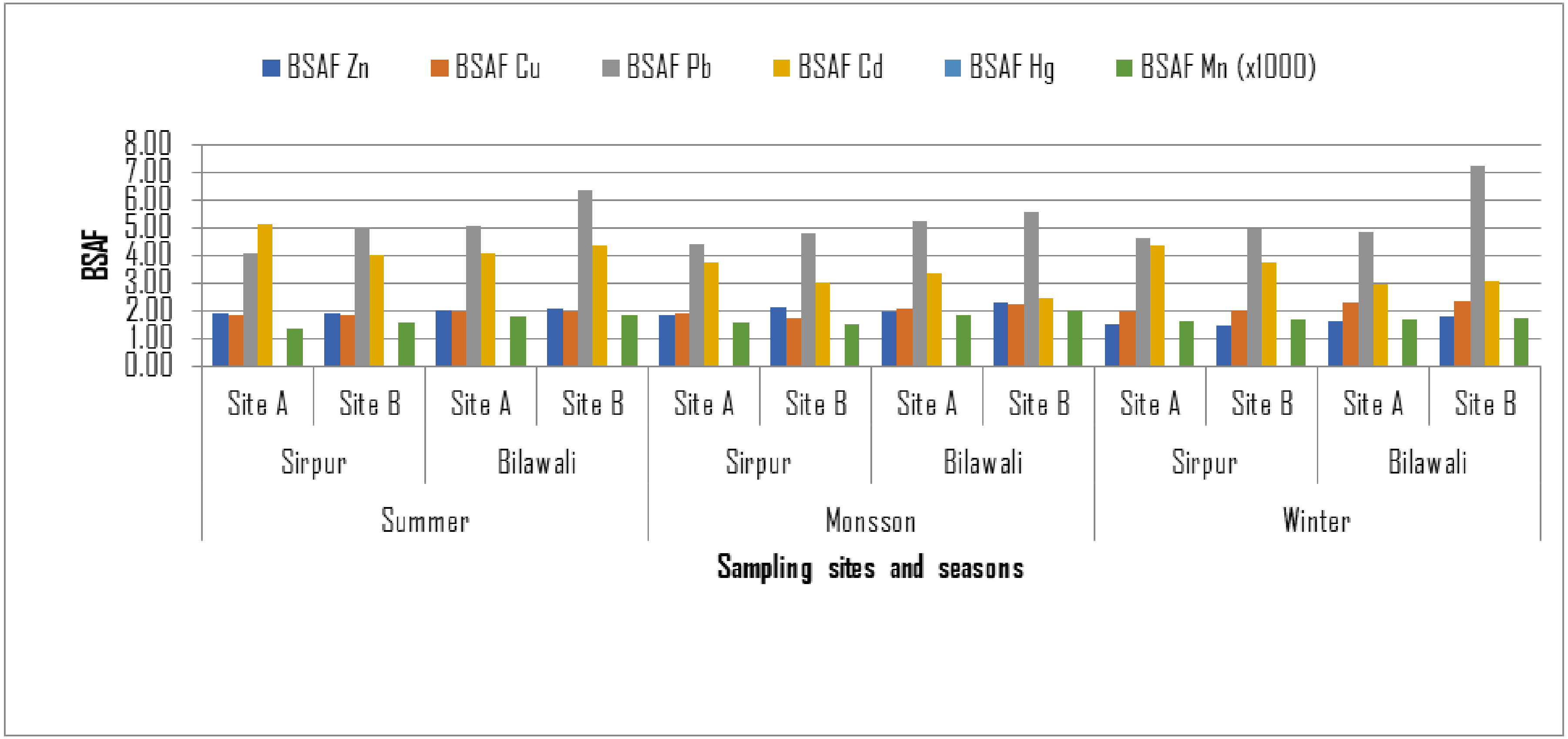


.3.3.2.5 BSAF (Bio-sediment accumulation factor)

Bio- sediment accumulation factor of different metal per individual with standard error in collected *Lamellidens marginali* bivalve samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 5. Bio- sediment accumulation factor of Zinc per individual with standard deviation in *Lamellidens marginali* bivalve samples collected from Sirpur Lake site A and site B were 1.94±0.12 and 1.95±0.19 in summer; 1.87±0.19 and 2.13±0.24 in monsoon; 1.52±0.12 and 1.49±0.17 in winter seasons whereas, Bilawali Tank site A and site B were reported 2.06±0.23 and 2.11±0.12 in summer; 1.99±0.15 and 2.34±0.16 in monsoon; 1.64±0.17 and 1.82±0.13 in winter seasons respectively. Bio- sediment accumulation factor of Copper per individual with standard deviation in *Lamellidens marginali* bivalve samples collected from Sirpur Lake site A and site B were 1.85±0.10 and 1.88±0.16 in summer; 1.90±0.20 and 1.78±0.11 in monsoon; 1.96±0.15 and 2.05±0.11 in winter seasons whereas, Bilawali Tank site A and site B were reported 1.98±0.21 and 1.97±0.22 in summer; 2.10±0.27 and 2.27±0.21 in monsoon; 2.29±0.13 and 2.36±0.16 in winter seasons respectively. Bio- sediment accumulation factor of Lead per individual with standard deviation in *Lamellidens marginali* bivalve samples collected from Sirpur Lake site A and site B were 4.12±2.14 and 5.04±2.22 in summer; 4.44±0.43 and 4.82±0.23 in monsoon; 4.65±0.29 and 4.97±0.22 in winter seasons whereas, Bilawali

Tank site A and site B were reported 5.10±2.10 and 6.40±0.24 in summer; 5.26±0.29 and 5.60±0.36 in monsoon; 4.85±0.13 and 7.23±0.18 in winter seasons respectively. Bio- sediment accumulation factor of Cadmium per individual with standard deviation in *Lamellidens marginali* bivalve samples collected from Sirpur Lake site A and site B were 5.17±3.45 and 4.01±1.06 in summer; 3.78±0.32 and 3.01±0.19 in monsoon; 4.36±0.18 and 3.74±0.020 in winter seasons whereas, Bilawali Tank site A and site B were reported 4.10±1.95 and 4.38±0.21 in summer; 3.36±0.10 and 2.49±0.21 in monsoon; 2.96±0.16 and 3.07±0.27 in winter seasons respectively.Bio- sediment accumulation factor of Cadmium per individual with standard deviation in *Lamellidens marginali* bivalve samples collected from Sirpur Lake site A and site B were 1.38± 0.03 and 1.58± 0.04 in summer; 1.59± 0.03 and 1.54± 0.03 in monsoon; 1.63± 0.04 and 1.71± 0.04 in winter seasons whereas, Bilawali Tank site A and site B were reported 1.81± 0.04 and 1.88± 0.05 in summer; 1.89± 0.04and 2.01± 0.05 in monsoon; 1.68± 0.03 and 1.76± 0.04 in winter seasons respectively.

Fig. 27. BSAF in *Lamellidens marginali* bivalves collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.3.3 *Unio* sp.

Table 6 presents the mean with standard deviation of collected observations of heavy metals in *Unio* sp samples.

Table 6. Seasonal variations of heavy metals accumulation in *Unio* sp. collected from Sirpur and Bilawali Tanks of Indore.

Parameters		Summer				Monsson				Winter			
	Metals	Sirpur		Bilawali		Sirpur		Bilawali		Sirpur		Bilawali	
		Site A	Site B	Site A	Site B	Site A	Site B	Site A	Site B	Site A	Site B	Site A	Site B
Dry weight whole body	Zn	1.24± 0.09	1.32± 0.10	1.15± 0.10	1.29± 0.12	1.19± 0.08	1.12± 0.08	1.05± 0.09	1.02± 0.07	1.05± 0.09	1.11± 0.08	1.14± 0.07	1.09± 0.07
	Cu	1.24± 0.09	1.32± 0.10	1.15± 0.10	1.29± 0.12	1.19± 0.08	1.12± 0.08	1.05± 0.09	1.02± 0.07	1.05± 0.09	1.11± 0.08	1.14± 0.07	1.09± 0.07
	Pb	1.24± 0.09	1.32± 0.10	1.15± 0.10	1.29± 0.12	1.19± 0.08	1.12± 0.08	1.05± 0.09	1.02± 0.07	1.05± 0.09	1.11± 0.08	1.14± 0.07	1.09± 0.07
	Cd	1.24± 0.09	1.32± 0.10	1.15± 0.10	1.29± 0.12	1.19± 0.08	1.12± 0.08	1.05± 0.09	1.02± 0.07	1.05± 0.09	1.11± 0.08	1.14± 0.07	1.09± 0.07
	Hg	1.24± 0.10	1.32± 0.11	1.15± 0.11	1.29± 0.13	1.19± 0.09	1.12± 0.09	1.05± 0.10	1.02± 0.08	1.05± 0.10	1.11± 0.09	1.14± 0.08	1.09± 0.08
	Mn	1.24± 0.11	1.32± 0.12	1.15± 0.12	1.29± 0.14	1.19± 0.10	1.12± 0.10	1.05± 0.11	1.02± 0.09	1.05± 0.11	1.11± 0.10	1.14± 0.09	1.09± 0.09
Metal concentration per unit body weight(µg/g)	Zn	451.26± 4.26	416.57± 5.02	341.76± 4.18	371.86± 4.29	309.89± 6.35	279.28± 6.52	253.41± 5.94	241.85± 5.26	349.28± 5.42	305.19± 5.34	289.67± 4.69	268.94± 3.97
	Cu	136.24± 2.12	112.45± 3.25	109.45± 2.96	92.59± 2.24	97.58± 3.02	85.49± 2.98	80.29± 3.01	72.49± 3.10	119.72± 3.24	98.71± 2.19	96.58± 2.05	86.58± 1.96
	Pb	103.52± 2.18	102.35± 2.69	96.57± 1.09	96.56± 1.12	94.53± 2.10	83.18± 2.48	73.54± 2.51	63.57± 2.09	102.51± 2.26	91.28± 2.08	83.49± 1.98	79.28± 1.68
	Cd	24.98± 0.39	18.68± 0.28	17.58± 0.23	15.48± 0.25	16.76± 0.12	11.49± 0.19	9.94± 0.21	7.29± 0.23	19.76± 0.34	13.81± 0.14	11.27± 0.13	10.68± 0.18
	Hg	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mn	32.21± 2.13	30.17± 2.01	25.43± 1.99	22.61± 1.89	30.13± 2.19	27.56± 1.86	19.65± 1.24	17.91± 1.56	31.11± 2.15	29.82± 1.97	20.18± 1.84	17.28± 1.39
Metal Body burden	Zn	559.47± 6.98	549.74± 6.41	392.89± 7.01	479.56± 6.41	368.63± 4.59	312.66± 5.12	265.94± 4.76	246.55± 4.61	366.60± 4.12	338.62± 5.34	330.09± 4.18	293.01± 3.85

(µg/individual)													
	Cu	168.80± 2.15	148.30± 2.39	125.73± 2.41	119.30± 2.29	115.98± 1.99	95.61± 1.87	84.17± 1.34	73.80± 1.41	125.57± 1.39	109.43± 1.42	109.97± 1.39	94.24± 1.21
	Pb	128.23± 3.12	134.97± 3.02	110.92± 2.95	124.43± 2.63	112.35± 1.87	93.03± 1.15	77.08± 1.22	64.71± 1.14	107.50± 1.26	101.18± 1.11	95.04± 1.06	86.28± 1.03
	Cd	30.84± 1.12	24.52± 1.24	20.08± 1.20	19.83± 0.27	19.81± 0.56	12.73± 0.34	10.30± 0.36	7.30± 0.21	20.61± 0.19	15.19± 0.18	12.71± 0.14	11.50± 0.14
	Hg	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mn	39.93± 3.25	39.81± 3.02	29.23± 2.56	29.15± 2.15	35.84± 3.11	30.85± 2.95	20.62± 1.84	18.25± 1.47	32.65± 3.24	33.08± 2.23	22.99± 2.11	18.82± 1.94
BWAF	Zn	3418.67± 65.48	3638.16± 66.54	3121.09± 62.54	3674.51± 60.27	3454.74± 60.14	3922.47± 51.12	4035.19± 53.29	4044.31± 48.65	2935.13± 42.36	2722.48± 33.48	2834.34± 31.28	2948.90± 34.52
	Cu	5538.21± 47.58	5354.76± 42.193	5527.78± 41.39	4723.98± 39.61	5843.11± 41.27	5737.58± 39.56	5818.11± 57.21	6358.77± 42.16	8034.89± 84.66	6208.18± 62.43	6396.03± 57.23	5696.05± 49.67
	Pb	3439.20± 39.26	3457.77± 35.46	3772.26± 33.27	4006.64± 18	3527.24± 55.29	1732.92± 51.02	3327.60± 38.16	3276.80± 35.48	5100.00± 61.38	3756.38± 46.35	3777.89± 38.51	3587.33± 34.27
	Cd	2904.65± 45.29	2364.56± 39.75	2441.67± 42.29	2310.45± 33.48	2327.78± 30.18	1665.22± 24.61	1483.58± 22.67	1278.95± 21.95	2600.00± 32.54	2001.45± 31.23	1587.32± 29.57	1547.83± 24.1
	Hg	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mn	3157.84± 50.29	3352.22± 46.27	3139.50± 49.82	3097.26± 47.53	3275.00± 37.26	3626.31± 35.14	2620.00± 33.48	2888.70± 37.24	3937.97± 28.53	4385.29± 33.29	2924.63± 34.29	2787.09± 28.69
BSAF	Zn	2.04± 0.29	1.97± 0.17	1.83± 0.12	2.17± 0.28	1.92± 0.15	2.00± 0.13	2.10± 0.17	2.29± 0.19	1.59± 0.11	1.50± 0.17	1.64± 0.13	1.80± 0.11
	Cu	1.96± 0.21	1.87± 0.19	2.01± 0.09	1.86± 0.15	1.90± 0.13	1.72± 0.09	2.07± 0.22	2.25± 0.20	2.01± 0.14	2.03± 0.13	2.29± 0.18	2.37± 0.13
	Pb	3.90± 0.39	4.85± 0.29	5.06± 0.24	6.39± 0.19	4.49± 0.28	5.04± 0.31	5.05± 0.37	5.64± 0.29	4.43± 0.22	4.74± 0.19	4.81± 0.19	4.06± 0.12
	Cd	5.33± 0.44	4.66± 0.23	4.49± 0.22	5.14± 0.17	4.67± 0.21	3.58± 0.14	3.30± 0.33	2.47± 0.24	4.93± 0.19	4.11± 0.17	3.25± 0.12	3.54± 0.11
	Hg	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mn (x1000)	1.11± 0.04	1.31± 0.03	1.33± 0.04	1.33± 0.03	1.43± 0.04	1.45± 0.03	1.22± 0.04	1.37± 0.05	1.35± 0.04	1.49± 0.05	1.18± 0.03	1.23± 0.03

3.3.3.1 Dry weight whole body

Dry weight whole body with standard error in collected *Unio* sp bivalve samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 6. Dry weight whole body with standard deviation in *Unio* sp bivalve samples collected from Sirpur Lake site A and site B were 1.24±0.09 and 1.32±0.10 in summer; 1.19±0.08 and 1.12±0.08 in monsoon; 1.05±0.09 and 1.11±0.08 in winter seasons whereas, Bilawali Tank site A and site B were reported 1.15±0.10 and 1.29±0.12 in summer; 1.05±0.09 and 1.02±0.07 in monsoon 1.14±0.07 and 1.09±0.07 in winter seasons respectively for all heavy metal accumulation.

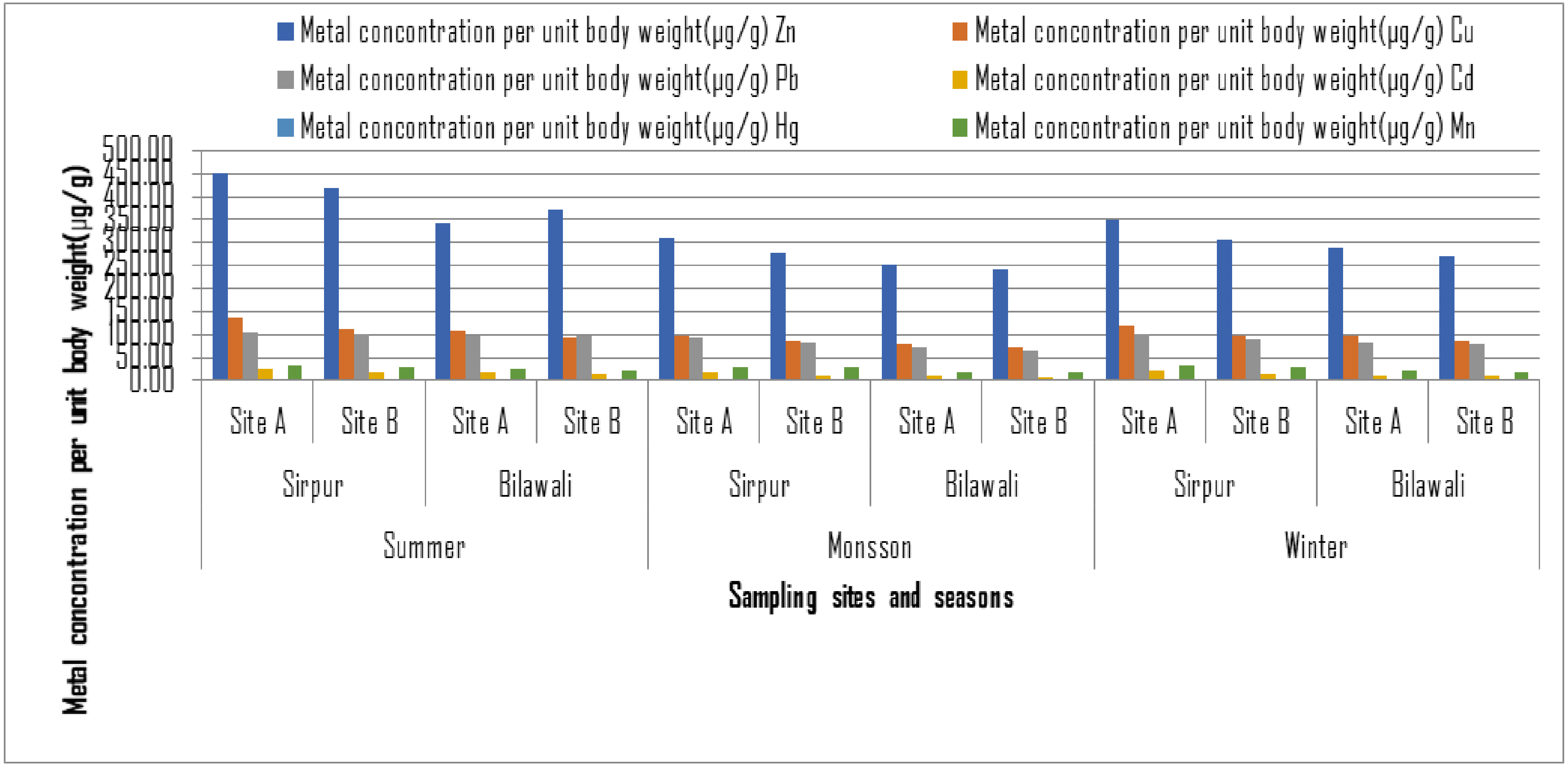
Fig. 28. Dry weight whole body of *Unio* sp bivalves collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.

3.3.3.2 Metal concentration per unit body weight (μg/g)

Metal concentration per unit body weight with standard error in collected *Unio* sp bivalve samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 6. Concentration of Zinc per unit body weight with standard deviation in *Unio* sp bivalve samples collected from Sirpur Lake site A and site B were 451.26±4.26 and 416.57±5.02 in summer; 309.89±6.35 and 279.28±6.52 in monsoon; 349.28±5.42 and 305.19±5.34 in winter seasons whereas, Bilawali Tank site A and site B were reported 341.76±4.18 and 371.86±4.29 in summer; 253.41±5.94 and 241.85±5.26 in monsoon; 289.67±4.69 and 268.94±3.97 in winter seasons respectively. Concentration of Copper per unit body weight with standard deviation in *Unio* sp bivalve samples collected from Sirpur Lake site A and site B were 136.24±2.12 and 112.45±3.25 in summer; 97.58±3.02 and 85.49±2.98 in monsoon; 119.72±3.24 and 98.71±2.19 in winter seasons whereas, Bilawali Tank site A and site B were reported 109.45±2.96 and 92.59±2.24 in summer; 80.29±3.01 and 72.49±3.10 in monsoon; 96.58±2.05 and 86.58±1.96 in winter seasons respectively. Concentration of Lead per unit body weight with standard deviation in *Unio* sp bivalve samples collected from Sirpur Lake site A and site B were 103.52±2.18 and 102.35±2.69 in summer; 94.53±2.10 and 83.18±2.48 in monsoon; 102.51±2.26 and 91.28±2.08 in winter seasons whereas, Bilawali Tank site A and site B were reported 96.57±1.09 and

96.56±1.12 in summer; 73.54±2.51 and 63.57±2.09 in monsoon; 83.49±1.98 and 79.28±1.68 in winter seasons respectively. Concentration of Cadmium per unit body weight with standard deviation in *Unio* sp bivalve samples collected from Sirpur Lake site A and site B were 24.98±0.39 and 18.68±0.28 in summer; 16.76±0.12 and 11.49±0.19 in monsoon; 19.76±0.34 and 13.81±0.14 in winter seasons whereas, Bilawali Tank site A and site B were reported 17.58±0.23 and 15.48±0.25 in summer; 9.94±0.21 and 7.29±0.23 in monsoon; 11.27±0.13 and 10.68±0.18 in winter seasons respectively. Concentration of Manganese per unit body weight with standard deviation in *Unio* sp bivalve samples collected from Sirpur Lake site A and site B were 32.21± 2.13 and 30.17± 2.01 in summer; 30.13± 2.19 and 27.56± 1.86 in monsoon; 31.11± 2.15 and 29.82± 1.97 in winter seasons whereas, Bilawali Tank site A and site B were reported 25.43± 1.99 and 22.61± 1.89 in summer; 19.65± 1.24 and 17.91± 1.56 in monsoon; 20.18± 1.84 and 17.28± 1.39 in winter seasons respectively

Fig. 29. Metal concentration per unit body weight in *Unio* sp bivalves collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.

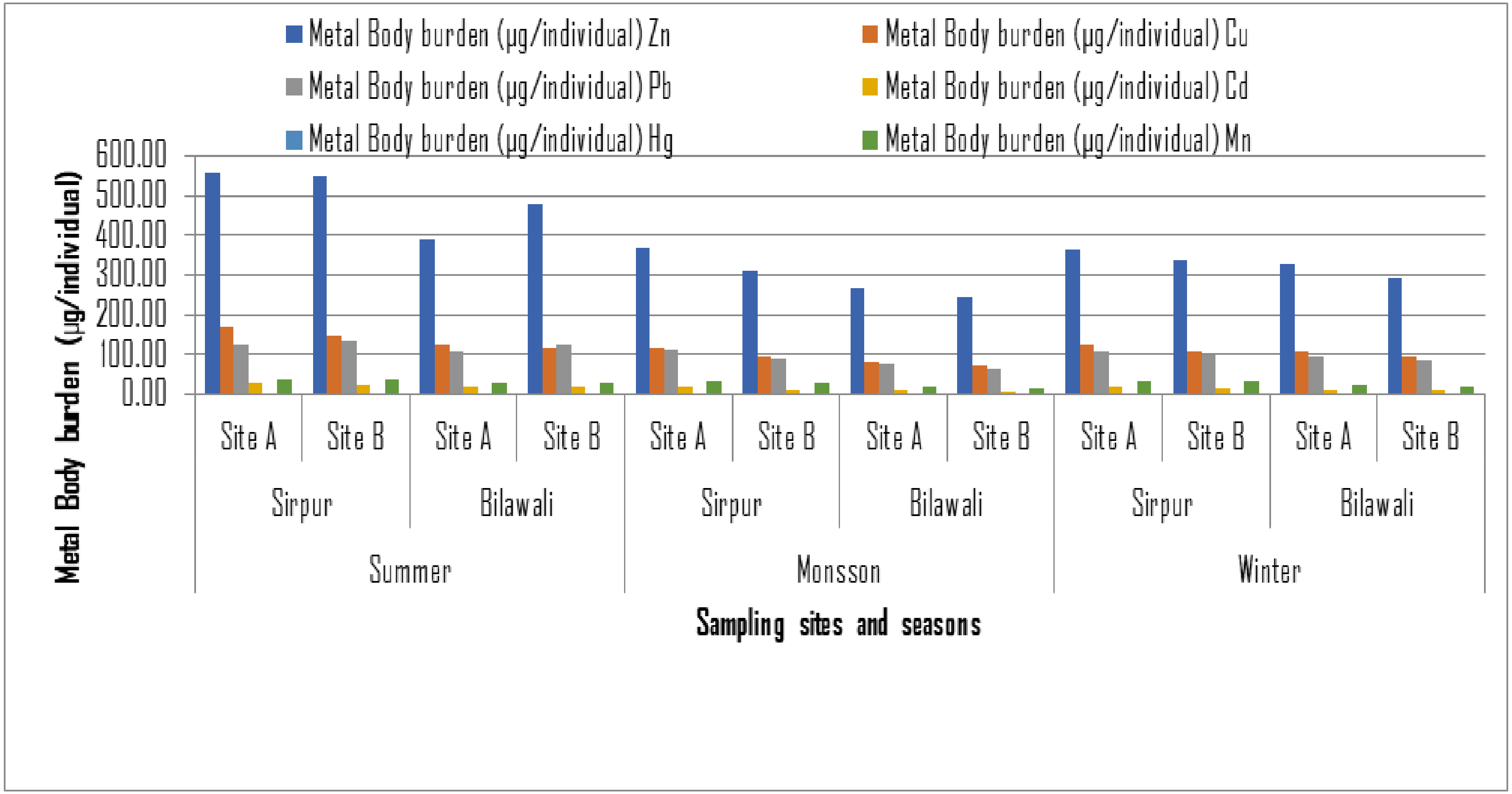


3.3.3.3 Metal Body burden (µg/individual)

Different metal body burden per individual with standard error in collected *Unio* sp bivalve samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 6. Metal body burden of Zinc per individual with standard deviation in *Unio* sp bivalve samples collected from Sirpur Lake site A and site B were 559.47±6.98 and 549.74±6.41 in summer; 368.63±4.59 and 312.66±5.12 in monsoon; 366.60±4.12 and 338.62±5.34 in winter seasons whereas, Bilawali Tank site A and site B were reported 392.89±7.01 and 479.56±6.41 in summer; 265.94±4.76 and 246.55±4.61 in monsoon; 330.09±4.18 and

293.01±3.85 in winter seasons respectively. Metal body burden of Copper per individual with standard deviation in *Unio* sp bivalve samples collected from Sirpur Lake site A and site B were 168.80±2.15 and 148.30±2.39 in summer; 115.98±1.99 and 95.61±1.87 in monsoon; 125.57±1.39 and 109.43±1.42 in winter seasons whereas, Bilawali Tank site A and site B were reported 125.73±2.41 and 119.30±2.29 in summer; 84.17±1.34 and 73.80±1.41 in monsoon; 109.97±1.39 and 94.24±1.21 in winter seasons respectively. Metal body burden of Lead per individual with standard deviation in *Unio* sp bivalve samples collected from Sirpur Lake site A and site B were 128.23±3.12 and 134.97±3.02 in summer; 112.35±1.87 and 93.03±1.15 in monsoon; 107.50±1.26 and 101.18±1.11 in winter seasons whereas, Bilawali Tank site A and site B were reported 110.92±2.95 and 124.43±2.63 in summer; 77.08±1.22 and 64.71±1.14 in monsoon; 95.04±1.06 and 86.28±1.03 in winter seasons respectively. Metal body burden of Cadmium per individual with standard deviation in *Unio* sp bivalve samples collected from Sirpur Lake site A and site B were 30.84±1.12 and 24.52±1.24 in summer; 19.81±0.56 and 12.73±0.34 in monsoon; 20.61±0.19 and 15.19±0.18 in winter seasons whereas, Bilawali Tank site A and site B were reported 20.08±1.20 and 19.83±0.27 in summer; 10.30±0.36 and 7.30±0.21 in monsoon; 12.71±0.14 and 11.50±0.14 in winter seasons respectively. Metal body burden of Manganese per individual with standard deviation in *Unio* sp. bivalve samples collected from Sirpur Lake site A and site B were 39.93± 3.25 and 39.81± 3.02 in summer; 35.84± 3.11 and 30.85± 2.95 in monsoon; 32.65± 3.24 and 33.08± 2.23 in winter seasons whereas, Bilawali Tank site A and site B were reported 29.23± 2.56 and 29.15± 2.15 in summer; 20.62± 1.84 and 18.25± 1.47 in monsoon; 22.99± 2.11 and 18.82± 1.94 in winter seasons respectively.

Fig. 30. Metal Body burden in *Unio* sp bivalves collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.

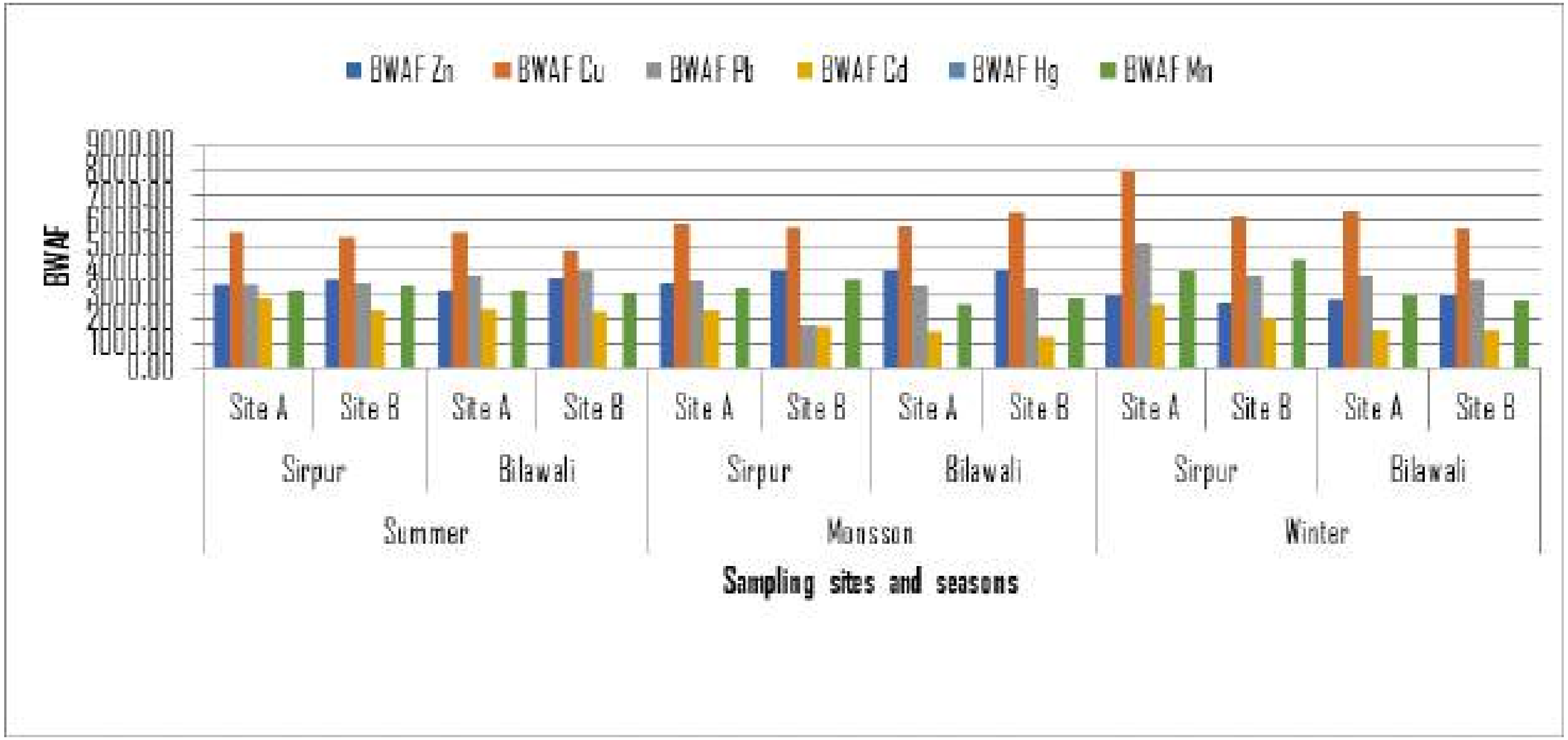


3.3.3.4 BWAF (Bio-water accumulation factor)

Bio-water accumulation factor of different metal per individual with standard error in collected *Unio* sp bivalve samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 6. Bio-water accumulation factor of Zinc per individual with standard deviation in *Unio* sp bivalve samples collected from Sirpur Lake site A and site B were 3418.67±65.48 and 3638.16±66.54 in summer; 3454.74±60.14 and 3922.47±51.12 in monsoon; 2935.13±42.36 and 2722.48±33.48 in winter seasons whereas, Bilawali Tank site A and site B were reported 3121.09±62.54 and 3674.51±60.27 in summer; 4035.19±53.29 and 4044.31±48.65 in monsoon; 2834.34±31.28 and 2948.90±34.52 in winter seasons respectively. Bio-water accumulation factor of Copper per individual with standard deviation in *Unio* sp bivalve samples collected from Sirpur Lake site A and site B were 5538.21±47.58 and 5354.76±42.193 in summer; 5843.11±41.27 and 5737.58±39.56 in monsoon; 8034.89±84.66 and 6208.18±62.43 in winter seasons whereas, Bilawali Tank site A and site B were reported 5527.78±41.39 and 4723.98±39.61 in summer; 5818.11±57.21 and 6358.77±42.16 in monsoon; 6396.03±57.23 and 5696.05±49.67 in winter seasons respectively. Bio-water accumulation factor of Lead per individual with standard deviation in *Unio* sp bivalve samples collected from Sirpur Lake site A and site B were 3439.20±39.26 and 3457.77±35.46 in summer; 3527.24±55.29 and

1732.92±51.02 in monsoon; 5100.00±61.38 and 3756.38±46.35 in winter seasons whereas, Bilawali Tank site A and site B were reported 3772.26±33.27 and 4006.64±18 in summer; 3327.60±38.16 and 3276.80±35.48 in monsoon; 3777.89±38.51 and 3587.33±34.27 in winter seasons respectively. Bio-water accumulation factor of Cadmium per individual with standard deviation in *Unio* sp bivalve samples collected from Sirpur Lake site A and site B were 2904.65±45.29 and 2364.56±39.75 in summer; 2327.78±30.18 and 1665.22±24.61 in monsoon; 2600.00±32.54 and 2001.45±31.23 in winter seasons whereas, Bilawali Tank site A and site B were reported 2441.67±42.29 and 2310.45±33.48 in summer; 1483.58±22.67 and 1278.95±21.95 in monsoon; 1587.32±29.57 and 1547.83±24.1 in winter seasons respectively. Bio-water accumulation factor of Manganese per individual with standard deviation in *Unio* sp bivalve samples collected from Sirpur Lake site A and site B were 3157.84± 50.29 and 3352.22± 46.27 in summer; 3275.00± 37.26 and 3626.31± 35.14 in monsoon; 3626.31± 35.14 and 4385.29± 33.29 in winter seasons whereas, Bilawali Tank site A and site B were reported 2441.67± 42.29 and 3097.26± 47.53 in summer; 3626.31± 35.14 and 2888.70± 37.24 in monsoon; 2924.63± 34.29 and 2787.09± 28.69 in winter seasons respectively.

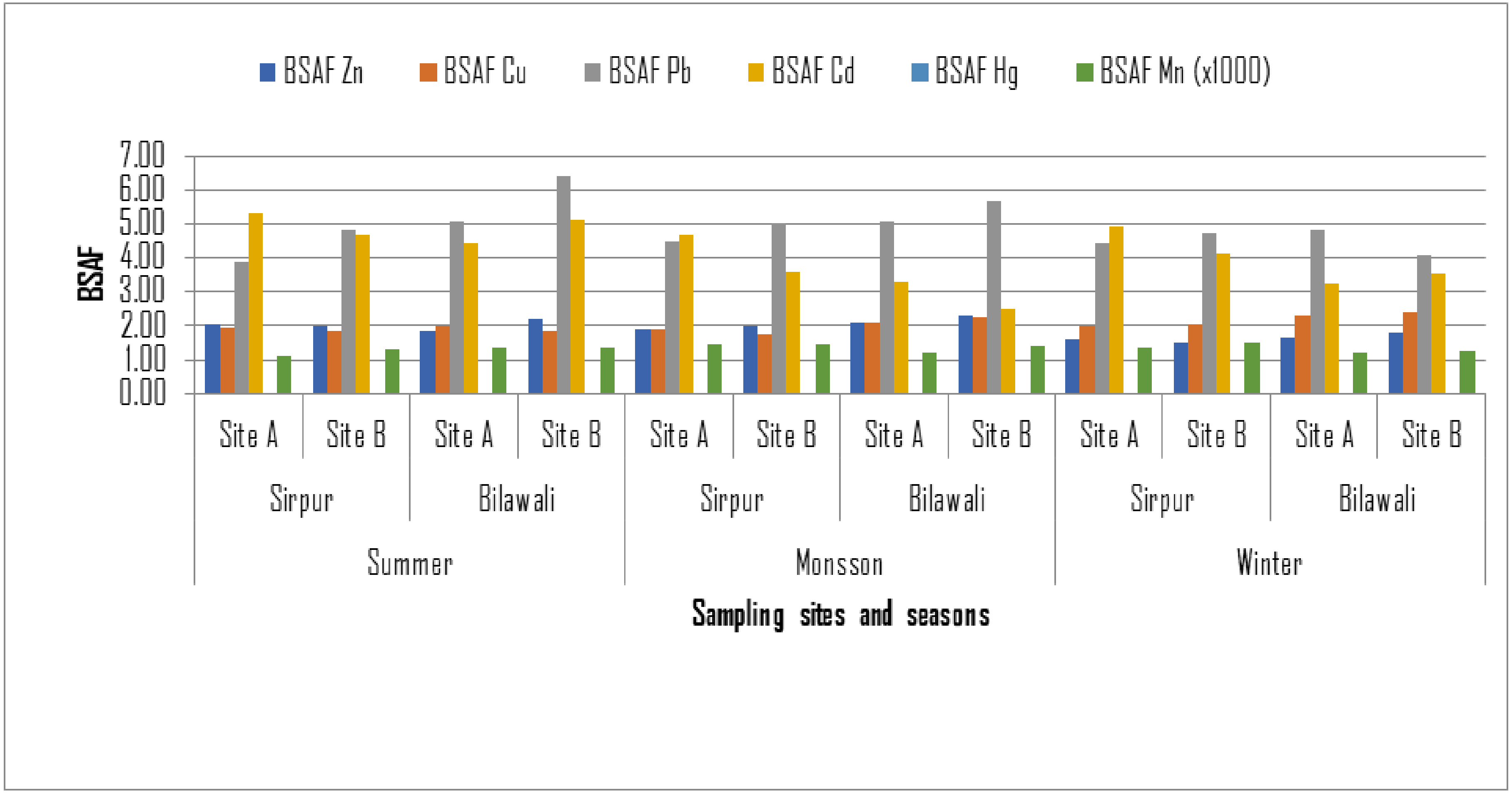
Fig. 31. BWAF in *Unio* sp bivalves collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.3.3.5 BSAF (Bio-sediment accumulation factor)

Bio- sediment accumulation factor of different metal per individual with standard error in collected *Unio* sp bivalve samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 6. Bio- sediment accumulation factor of Zinc per individual with standard deviation in *Unio* sp bivalve samples collected from Sirpur Lake site A and site B were 2.04±0.29 and 1.97±0.17 in summer; 1.92±0.15 and 2.00±0.13 in monsoon; 1.59±0.11 and 1.50±0.17 in winter seasons whereas, Bilawali Tank site A and site B were reported 1.83±0.12 and 2.17±0.28 in summer; 2.10±0.17 and 2.29±0.19 in monsoon; 1.64±0.13 and 1.80±0.11 in winter seasons respectively. Bio- sediment accumulation factor of Copper per individual with standard deviation in *Unio* sp bivalve samples collected from Sirpur Lake site A and site B were 1.96±0.21 and 1.87±0.19 in summer; 1.90±0.13 and 1.72±0.09 in monsoon; 2.01±0.14 and 2.03±0.13 in winter seasons whereas, Bilawali Tank site A and site B were reported 2.01±0.09 and 1.86±0.15 in summer; 2.07±0.22 and 2.25±0.20 in monsoon; 2.29±0.18 and 2.37±0.13 in winter seasons respectively. Bio- sediment accumulation factor of Lead per individual with standard deviation in *Unio* sp bivalve samples collected from Sirpur Lake site A and site B were 3.90±0.39 and 4.85±0.29 in summer; 4.49±0.28 and 5.04±0.31 in monsoon; 4.43±0.22 and 4.74±0.19 in winter seasons whereas, Bilawali Tank site A and site B were reported 5.06±0.24 and 6.39±0.19 in summer; 5.05±0.37 and 5.64±0.29 in monsoon; 4.81±0.19 and 4.06±0.12 in winter seasons respectively. Bio- sediment accumulation factor of Cadmium per individual with standard deviation in *Unio* sp bivalve samples collected from Sirpur Lake site A and site B were 5.33±0.44 and 4.66±0.23 in summer; 4.67±0.21 and 3.58±0.14 in monsoon; 4.93±0.19 and 4.11±0.17 in winter seasons whereas, Bilawali Tank site A and site B were reported 4.49±0.22 and 5.14±0.17 in summer; 3.30±0.33 and 2.47±0.24 in monsoon; 3.25±0.12 and 3.54±0.11 in winter seasons respectively. Bio- sediment accumulation factor of Manganese per individual with standard deviation in *Unio* sp bivalve samples collected from Sirpur Lake site A and site B were 1.11± 0.04 and 1.31± 0.03 in summer; 1.43± 0.04 and 1.45± 0.03 in monsoon; 1.35± 0.04 and 1.49± 0.05 in winter seasons whereas, Bilawali Tank site A and site B were reported 1.33± 0.04 and 1.33± 0.03 in summer; 1.22± 0.04 and 1.37± 0.05 in monsoon; 1.18± 0.03 and 1.23± 0.03 in winter seasons respectively

Fig. 32. BSAF in *Unio* sp bivalves collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.4 Oxidative stress biomarker studies in bivalves:

Glutathione peroxidase, Superoxide dismutase, Glutathione-S-transferase, Reduced glutathione, Catalase and Lipid Peroxidation parameters of oxidative stress biomarkers were assessed in *Lamellidens corrianus*, *Lamellidens marginali* and *Unio* sp bivalves samples collected from two different sites of Sirpur and Bilawali Tanks, Indore in summer, monsoon and winter seasons and presented in table 7-9.

3.4.1 *Lamellidens corrianus*

Table 7 presents the mean with standard deviation of collected observations of oxidative biomarkers in *Lamellidens corrianus* samples.

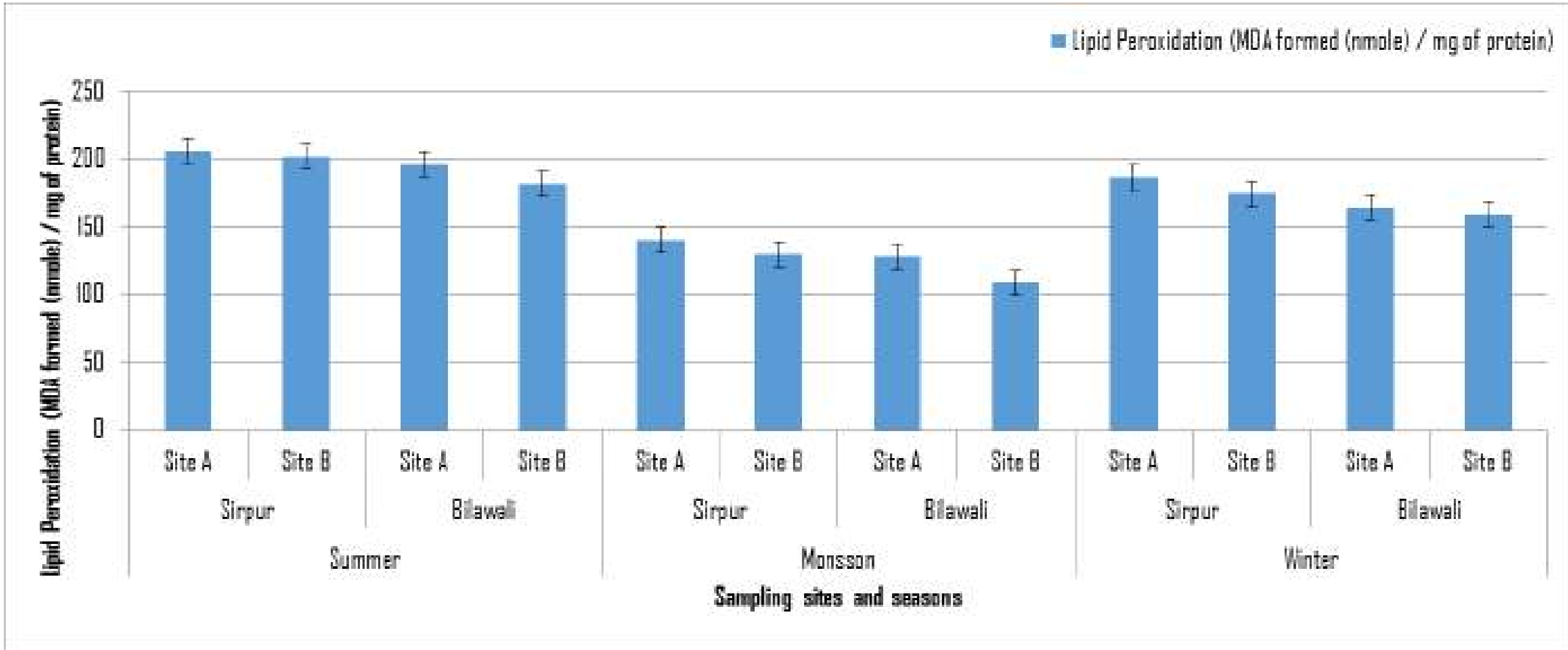
Table 7. Seasonal variations of oxidative stress in *Lamellidens corrianus* collected from Sirpur and Bilawali Tanks of Indore.

Parameters	**Summer**				**Monson**				**Winter**			
	Sirpur		**Bilawali**		**Sirpur**		**Bilawali**		**Sirpur**		**Bilawali**	
	Site A	**Site B**	**Site A**	**Site B**	**Site A**	**Site B**	**Site A**	**Site B**	**Site A**	**Site B**	**Site A**	**Site B**
Lipid Peroxidation	205.41±4.29	201.46±4.12	195.62±5.21	181.4±5.14	139.87±4.02	129.76±3.87	128.12±3.89	108.76±2.76	186.24±3.76	174.25±3.29	164.24±3.54	159.27±3.69
Glutathione-S-transferase	222.15±4.95	209.48±3.69	202.82±4.86	201.42±4.20	143.76±3.24	133.49±3.43	122.54±3.29	114.25±3.02	195.48±2.94	176.24±2.76	156.35±3.02	154.38±2.96
Reduced glutathione	6.54±0.14	8.01±0.13	11.59±0.21	13.58±0.21	9.02±0.18	9.24±0.14	13.09±0.13	16.28±0.11	8.01±0.09	9.01±0.11	12.29±0.12	16.24±0.17
Superoxide dismutase	119.84±1.29	125.64±1.39	135.47±2.58	143.57±1.97	149.84±1.92	169.24±2.09	190.18±2.16	201.26±2.84	128.36±1.28	151.31±1.34	149.75±1.19	160.75±1.17
Catalase	92.35±1.14	93.27±1.18	103.52±2.13	104.26±1.96	131.06±1.84	132.56±1.79	139.86±1.29	152.45±1.09	116.24±1.16	122.58±1.29	124.51±1.05	128.36±1.76
Glutathione peroxidase	33.75±0.26	37.45±0.23	37.19±0.22	41.79±0.16	50.42±0.17	52.01±0.14	53.18±0.11	60.01±0.14	46.59±0.11	49.78±0.11	50.14±0.09	50.28±0.08

3.4.1.1 Lipid peroxidation

Lipid peroxidation with standard error per mg protein in collected *Lamellidens corrianus* bivalve samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 7. Lipid peroxidation with standard deviation per mg protein in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 205.41±4.29 and 201.46±4.12 in summer; 139.87±4.02 and 129.76±3.87 in monsoon; 186.24±3.76 and 174.25±3.29 in winter seasons whereas, Bilawali Tank site A and site B were reported 195.62±5.21 and 181.4±5.14 in summer; 128.12±3.89 and 108.76±2.76 in monsoon; 164.24±3.54 and 159.27±3.69 in winter seasons.

Fig. 33. Lipid peroxidation activity in *Lamellidens corrianus* bivalves collected from different sampling sites of Sirpur and Bilawali Tanks of Indore in different seasons.



3.4.1.2 Glutathione-S-transferase

Glutathione-S-transferase with standard error per mg protein in collected *Lamellidens corrianus* bivalve samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 7. Glutathione-S-transferase with standard deviation per mg protein in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 222.15±4.95 and 209.48±3.69 in summer; 143.76±3.24 and 133.49±3.43 in monsoon; 195.48±2.94 and 176.24±2.76 in winter seasons whereas, Bilawali Tank site A and site B were reported 202.82±4.86 and 201.42±4.20 in summer; 122.54±3.29and 114.25±3.02 in monsoon; 156.35±3.02 and 154.38±2.96 in winter seasons respectively.

3.4.1.3 Reduced glutathione

Reduced glutathione with standard error per mg protein in collected *Lamellidens corrianus* bivalve samples of Sirpur and Bilawali Tank in summer, monsoon and winter seasons is shown in Table 7. Reduced glutathione with standard deviation per mg protein in *Lamellidens corrianus* bivalve samples collected from Sirpur Lake site A and site B were 6.54±0.14 and 8.01±0.13 in summer; 9.02±0.18 and 9.24±0.14 in monsoon; 8.01±0.09 and 9.01±0.11 in winter seasons whereas, Bilawali Tank site A and site B were reported 11.59±0.21 and