The Impact Of Ascorbic Acid On The Bioaccumulation Of Zinc Sulphate In Freshwater Bivalve Lamelliden's Corrianus

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Abstract:
This study delves into the impact of ascorbate on the bioaccumulation of zinc sulphate in bivalves, such as clams and mussels. These bivalves are notorious for absorbing heavy metals from their surroundings, which can pose threats to ecosystem health and human consumption. Zinc sulphate, a prevalent industrial pollutant, can build up in bivalve tissues, potentially causing harm to both the bivalves themselves and organisms higher up in the food chain.
Ascorbate, also known as vitamin C, is renowned for its antioxidant properties and its ability to counteract heavy metal toxicity in various organisms. In this study, bivalves were exposed to zinc sulphate with and without the presence of ascorbate, and zinc tissue concentrations were measured. The results revealed that supplementing with ascorbate significantly decreased the bioaccumulation of zinc sulphate in bivalves compared to untreated controls. Additionally, ascorbate seemed to alleviate the oxidative stress caused by zinc exposure, indicating a protective role against heavy metal toxicity.
These findings emphasize the potential of ascorbate as a natural remedy for heavy metal pollution in aquatic environments. They also underscore the importance of further research into the mechanisms of action and ecological implications of ascorbate in combating heavy metal toxicity.

Key Words: Ascorbate, Heavy metal, Toxicology, Bivalve.

Introduction

Metals are naturally found on Earth, but human activities have caused a big change in how they move around and affect living things. In places like estuaries, which are where rivers meet the ocean, metals can come from things like rocks breaking down, pollution in the air, mining, farming, and factories (31). This extra metal from human activities, especially since the Industrial Revolution, has made the water in estuaries dirty and harmful to the animals that live there (42). One example of sediment-dwelling marine fauna is bivalves, which are meiofaunal organisms that reside in the upper layers of sediment. As a result, the sediment-water interface plays a crucial role in their habitat. These organisms possess the capacity to take in metals through both water and food sources, including suspended matter and sediment found at the interface of water and sediment. The feeding process of bivalves involves filtering water through their gills (19) and, therefore, can take the metals from the dissolved phase, particulate phase, and sediment. Metals, especially when present in a labile form in sediments, can be easily absorbed by bivalves. While trace amounts of iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) are essential for the metabolism of bivalves, they can become toxic when present in higher concentrations (35). Bivalves are valuable indicators of pollution potential and recent contamination levels (29). Trace metals in bivalves can have detrimental effects such as stunted growth, disruptions in metabolic processes, and increased susceptibility to diseases (18). Metals can enter the food chain, including humans, through bivalves, which serve as the primary consumers in the estuarine ecosystem (13). Furthermore, bivalves are a nutritious source of proteins, minerals, and vitamins, making them a popular choice for seafood among humans (32). According to Miloskovic (27), high levels of metal content in bivalves can have negative effects on human health. Therefore, it is crucial to regularly monitor the metal content in bivalves to ensure the safety of consumers. Numerous metals are discharged into freshwater from both natural and anthropogenic sources. Some of the most significant metals in terms of water pollution are zinc (Zn), copper (Cu), lead (Pb), cadmium (Cd), mercury (Hg), nickel (Ni), and chromium (Cr). While copper, nickel, chromium, and zinc are essential trace metals for living organisms, they can become toxic at elevated levels. On the other hand, metals like arsenic (As), mercury (Hg), cadmium (Cd), copper (Cu), chromium (Cr), manganese (Mn), and zinc (Zn) do not degrade. Metal ions accumulate throughout the food chain, posing serious health risks even at very low levels. These risks include reduced growth and development, cancer, organ damage, and nervous system damage. In extreme cases, metal ions can have severe consequences. Traditionally, water quality monitoring for metals has been conducted by analyzing the concentration of metals in both water and sediments. Unfortunately, research has shown that this method may yield inaccurate results (16). Misleading decision-making in water quality assessment is a common issue. To address this problem, many researchers utilize benthic organisms as indicators of pollutant levels and long-term impacts within an ecosystem. Metal accumulation in living organisms can result in concentrations several orders of magnitude higher than those found in the surrounding water (10). Many different types of freshwater creatures have lead accumulation in their tissue, which makes lead a cumulative toxicant (24).

According to Teodorovic (40) and Abdullah (1), studies on metals in aquatic biota suggest that these organisms may serve as more reliable indicators of water quality than traditional chemical analyses of water columns and sediment.

Ascorbic acid, commonly known as Vitamin C, is an essential component for the normal development of most species. It is produced internally, giving it a hormone-like characteristic. Glucose and other hexoses can be converted into glucose, which is the initial material for the biosynthesis of ascorbic acid. This vital nutrient plays a crucial role in tissue synthesis, growth processes, and rapid tissue repair during trauma or disease conditions. Ascorbic acid is essential for the synthesis of tissue collagen and the development of red blood cells (39). Furthermore, research conducted by Chatterjee (11) has shown that ascorbic acid plays a vital role in protecting mammalian tissues from oxidative damage, both intracellularly and extracellularly. The essential nutrient, vitamin C, is significantly influenced and altered by a variety of environmental pollutants, including pesticides (3,5,17).

Numerous studies indicate that bivalves are the ideal experimental model for studying detoxification mechanisms and the impacts of detoxifying agents. However, there is a lack of research on the levels of ascorbic acid in the tissue of freshwater bivalve mollusks. Given the importance of ascorbic acid and the limited information on its concentration in freshwater bivalve tissues and its potential role in reducing metal toxicity, this study was initiated.

MATERIALS AND METHODS:

Lamellidens corrianus, a species of bivalve, were gathered from Jayakwadi Dam in Paithan, situated 60 km from Aurangabad in Maharashtra State. In order to create ideal conditions for experimentation, the bivalves were acclimated in dechlorinated tap water for five days within a laboratory environment. After acclimatization, the robust and active bivalves were divided into three groups: A, B, and C. Group A functioned as the control, while group B was subjected to a chronic concentration of $ZnSO_4$ (8.143 ppm) with an LC50 value of 96 hr/10. Group C was exposed to a chronic concentration of $ZnSO_4$ (LC50 value of 96/hr/10) along with 50 mg/L of ascorbic acid for a duration of 20 days.

Following a 20-day exposure to heavy metals, the bivalves in group B were further divided into two subgroups, D and E. The entire body mass of bivalves from groups A, B, and C was collected after 10 and 20 days, while that of groups D and E was collected after 3, 6, and 9 days.

While the bivalves in group E were exposed to 50 mg/L of ascorbic acid for a period of 9 days, the bivalves in group D were allowed to naturally self-cure in normal water. The samples were dried at 80°C in an oven until a constant weight was achieved. Subsequently, they were carefully stored in air-tight specimen bottles, with the cork sealed with wax to ensure optimal preservation. The 500 mg sample of whole-body tissue was digested in a 10 ml acid mixture (HCl: HNO_3 in a 3:1 ratio) on a hot plate until completely dry. Following digestion, the samples were mixed with double distilled water and cooled in a water bath for 6-7 hours. Once cooled, the samples were filtered using Whatman grade 541 filter paper. The resulting solution was then diluted to a final volume of 50 ml using double glass-distilled water in a volumetric flask. The Zinc content was determined by analysing the prepared samples using an Atomic Absorption Spectrophotometer (Chemito). It is crucial to emphasize that meticulous filtration and dilution techniques were utilized to guarantee precise and dependable results. The utilization of top-notch filter paper and double distilled water further aided in reducing any possible sources of contamination. In conclusion, this method offers a precise and effective approach to analysing zinc content in a variety of samples.

OBSEREVATION AND RESULTS:

Table 1.1 presents a summary of the $ZnSO_4$ content in Lamellidens corrianus following exposure to zinc sulphate(8.143ppm) with and without ascorbic acid, as well as during the recovery period. The findings suggest that the concentration of bioaccumulated Zinc in the soft tissue of the bivalves was significantly elevated in the presence of $ZnSO_4$ (8.143ppm). After 10 days of exposure, the bioaccumulated Zinc in the presence of $ZnSO_4$ was measured at 185.13µg/gm, which then increased to 244.4µg/gm after 20 days. Interestingly, the rate of bioaccumulation was found to be lower in bivalves exposed to zinc sulphate with ascorbic acid compared to those exposed to zinc sulphate alone. Specifically, after 10 days of exposure to $ZnSO_4$, the bioaccumulated zinc was recorded at 126.03µg/gm, and after 20 days, it had risen to 163.07 µg/gm. These findings suggest that the presence of $ZnSO_4$ may play a role in the bioaccumulation of zinc in bivalves. Interestingly, bivalves that were pre-exposed to $ZnSO_4$ showed faster recovery in the presence of ascorbic acid compared to those that were allowed to naturally cure. After 3 days of recovery, the bioaccumulated zinc levels decreased to 183.95 µg/gm in normal water and 165.35µg/gm in the presence of ascorbic acid. By day 6, the levels dropped further to 153.07µg/gm and 108.03 µg/gm, respectively. After 9 days, the levels were significantly reduced to 114.03µg/gm in normal water and 54.53µg/gm in the presence of ascorbic acid.

In conclusion, the findings indicate that the presence of ascorbic acid can enhance the recovery of bivalves exposed to Zinc Sulphate. Furthermore, bivalves exposed to Zinc Sulphate with ascorbic acid exhibit a lower rate of bioaccumulation of Zinc.

These results have significant implications for the management of aquatic ecosystems and the protection of aquatic organisms from metal toxicity.

Table No. 1.1 Zinc content (µg/gm dry weight) in Whole body of *Lamellidens corrianus* after chronic exposure to Zn without, with Ascorbic acid and during recovery

Treatment	Zn content (µg/gm dry weight)				
	10 days	20 days	3 days	6 days	9 days
(A) Control	0.1240	0.2347	-	-	-
(B) $ZnSO_4$	185.13 (-1392.9838)	244.41 (-8517.4528)	-	-	-
(C) $ZnSO_4$ + Ascorbic acid (50mg/L)	126.03 (-916.3709)	163.07 (-1110.61)	-	-	-
(D) Normal water (Recovery)	-	-	183.95 (-65.1456)	153.07 (-37.3690)	114.33 (-53.2201)
(E) Normal water + Ascorbic acid (50 mg/L) (Recovery)	-	-	165.35 (-32.3445)	108.03 (-55.7978)	54.53 (-77.6882)

Discussion:

The tremendous affinity that heavy metals have for attaching to biological molecules is one of their distinguishing characteristics. Many scientists have taken an interest in this particular characteristic because heavy metals are known to be stable in the environment and to have the capacity to have harmful consequences for an extended period of time. Preserving environmental safety requires an understanding of the distribution and accumulation of heavy metals in various animal tissues. Concern over the bioaccumulation of heavy metals in mollusks has grown recently since these aquatic creatures may suffer negative consequences from high concentrations of these heavy metals. Consequently, studying how heavy metals affect biological systems is an important field of study with consequences for public health and environmental preservation.

Semi-permeable membranes found in various organs, including the gastrointestinal system, mantle, gills, and mouth lining, allow metal ions to enter the body. The concentration of metals in the water affects how much of them aquatic organisms absorb. But these substances can only build up if the rate of absorption outpaces the rate of excretion.

Toxic compounds can accumulate in different ways in different organs, and the body's distribution of these poisons might change over time. Recent research has demonstrated that the tissues of organisms exposed to both field and laboratory environments exhibit distinct patterns of heavy metal distribution, storage, and binding. Muscle, visceral mass, mantle, gills, and labial palps were shown to be the most damaged, in decreasing order of distribution (25). The fish When it comes to zinc buildup, Catla catla has a higher level than other fish species. The fast absorption of zinc into the cell membrane is highlighted in the kinetics of zinc uptake reports by Nair (30) and Singh and Gaur (36,37). Diffusion then regulates the rate of uptake and binds to proteins within the cell. Throughout the fish's growth cycle, this process takes place. The effect of water hardness on zinc toxicity to Gambusia affinis was shown in a study by Kallanagoudar and Patil (20). Furthermore, freshwater fishes have minimal levels of zinc bioaccumulation, according to Fatma (12).

Further studies using mathematical analysis by Busch et (8) shown the significant impact of seasonal fluctuations, dissolved oxygen, and salinity on the bioavailability of heavy metals such Pb, Cd, Cr, Ni, Zn, and Cu. It was also found that the physico-chemical properties of the water can affect the bioaccumulation potential of these metals in any organism.

Elevated levels of manganese, chromium, cadmium, lead, iron, arsenic, copper, nickel, lead, zinc etc. were found in hepatopancreas of snails compared to the fish liver (43) and in Ruditapes decussatus and Ruditapes philippinarum (41) in the same area.

Adami (2) developed a model predicting the labile percentages of Cu, Pb, Cd, and Zn in sediments based on the mass and length of the benthic bivalve Corbicula gibbasa, which they used as a bioindicator. Comparing B. pharaonis molluscs to P. radiata, Boyden (7) found that the former showed higher accumulation of trace metal concentrations and body size, with smaller organisms displaying more accumulation. Smaller animals' higher metabolic rates may be the cause of their higher bioaccumulation rate. Michiel (26) brought attention to the effects of zinc and lead on Dreissena polymorpha filtration rate, which rose with longer exposure times. The concentration of metal in the water, the amount of metal accumulated by the mussels, or both, were associated with a decrease in filtering rate. The snail Viviparous bengalensis had the highest quantity of zinc (0.3284 mg/gm dry weight). Because of their benthic environment and detritus-feeding habits, chironomid larvae also showed a higher buildup of zinc (28).

Zinc is distributed in a way that suggests it mostly builds up through food ingestion, with the alimentary tract having the highest concentrations. The buildup of zinc was found to follow this pattern: gastrointestinal tract > gills > kidney > liver > muscle. On the other hand, according to Rao (34), the pattern for lead accumulation was gill > kidney > alimentary tract > liver > muscle. It is crucial to recognize that even minimal levels of heavy metals can pose a threat to our health, underscoring the importance of detoxification. (4,6, 21).
It is crucial to take proactive measures for detoxification in order to lessen the negative effects of zinc poisoning. This could entail adopting methods to remove zinc from the body and reducing exposure to it. We can protect our environment and ourselves from the harmful effects of zinc by adopting these preventative steps.

Ascorbic acid, sometimes referred to as vitamin C, is an essential nutrient that is involved in the body's distribution and excretion of hazardous metals and trace minerals (22). Ascorbic acid's effects on these elements have been studied by many researchers. According to Hughes' (15) research, ascorbic acid helps preserve the -SH group of proteins, which is crucial for metal interaction, by acting as a diffusible biological antioxidant at the right concentration. Strong antioxidant L-ascorbic acid can intensify its defences by chelating metals or interacting with free radicals to drive them out of the body (38). The synthesis and maintenance of collagen, the building block of connective tissue that makes up the skin, ligaments, cartilage, vertebral discs, joint linings, capillary walls, bones, and teeth, is one of ascorbic acid's most important functions. Collagen and vitamin C work together to give the body stability and structure, promote wound healing, and preserve healthy blood vessels. Furthermore, vitamin C has a role in the metabolism of tyrosine to dopamine and adrenaline, folic acid to its active form tetrahydro folic acid, and tryptophan to serotonin, as well as other hazardous metals. In addition to being a potent antioxidant vitamin, ascorbic acid is a great reducing agent. It has the capacity to lower metals like copper and iron and can act as a donor antioxidant in the oxidation process brought on by free radicals (9). It's interesting to note that ascorbic acid can also act as a prooxidant. Hill (14) discovered that ascorbic acid might lessen growth retardation in chicks that is brought on by cadmium and other hazardous metals.
Moreover, ascorbic acid can combine with other metals to generate salts that lower its action. For instance, sperm production in the cauda epididymis was dramatically decreased after 60 days of methyl mercury feeding, but it restored after 60 days of feeding mercury coupled with ascorbic acid, according to a study by Rao (33). Furthermore, in the bivalve Corbicula striatella, Mahajan and Zambare (23) found that ascorbic acid can aid in the recovery of protein lost as a result of copper and mercury.
Ascorbic acid is essential for the metabolism of proteins, carbohydrates, or both because it is a hydrogen carrier. It additionally aids in keeping blood vessels strong during toxicosis.
Furthermore, ascorbic acid acts as an antioxidant, aids in detoxification, and contributes to collagen synthesis. These functions provide protection against damage caused by heavy metals, aid in recovery from heavy metal-induced damage, and reduce the bioaccumulation of zinc in the body

Conclusion:
The current study has found that the administration of ascorbic acid can effectively reduce the bioaccumulation of Zn. This is due to the chelating activity of ascorbic acid, which binds to the metal or free radical and causes them to precipitate. Once these free radicals have been precipitated, they are excreted by the bivalve.

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