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Review

# Molluscs as Experimental Models for the Study of Mucomics

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**Abstract:** Mucomics is the study of mucus and its biochemical properties. This discipline has gained increasing attention due to the critical roles mucus plays in protection, adhesion, and communication across species. Ethical restriction on vertebrate research have driven interest in invertebrate models such as molluscs. Molluscs produce a large amount of mucus which several functions including immune defense, digestion, and environmental adaptation. Molluscs - terrestrial, freshwater, or marine - are valuable models for investigating mucus composition and its responses to environmental stressors, including heavy metal contamination. Histochemical and glycomic techniques have revealed variations in mucin glycosylation patterns that influence mucus functionality, such as its viscoelastic and adhesive properties. Bivalves, widely used as bioindicators, accumulate pollutants in their mucus, reflecting environmental health. Investigative techniques such as lectin histochemistry, proteomic, and glycomic analyses provide insights into the impact of contaminants on mucus composition. Further research on molluscan mucins can enhance understanding of their physiological roles, environmental interactions, and potential biomedical applications. By integrating molecular and histochemical approaches, mucomics studies offer a comprehensive perspective on mucus function, advancing both ecological monitoring and biotechnological applications.

**Keywords:** Bioindicators; Environmental contaminants; Molluscan mucus; Histochemistry

## 1. Introduction

The study of mucus and its biochemical properties, collectively referred to as mucomics, has gained increasing interest due to the biological significance of mucus in protection, adhesion, and communication in many species.

In recent decades, histochemical and glycohistochemical studies have characterized the mucins of various organisms, also vertebrates [1-4], with the aim of elucidating the biological and functional roles of mucus across different taxa and identifying potential similarities with humans. Nevertheless, the pursuit of experimental and applied models must necessarily consider both ethical constraints and housing requirements.

The European Parliament's Directive 2010/63/EU on the protection of animals used for scientific purposes, implemented in Italy through D.Lgs. 26/2014, has strongly restricted animal experimentation to promote animal welfare and minimize suffering. This legislation applies to (1)

live non-human vertebrates, including larval forms capable of independent feeding and mammalian fetuses in the final third of their development, and (2) cephalopod molluscs.

As a result of these regulations, the use of animals such as fish (e.g., zebrafish *Danio rerio*), amphibians (e.g., *Xenopus laevis*), mice, and rats in scientific research is highly controlled. These animals must be registered, housed in isolated enclosures at research facilities, and cared for by trained technicians responsible for maintaining their health and hygiene. Consequently, obtaining permits for the housing and use of these animals is challenging, as such research in Italy requires dedicated facilities, qualified personnel, and adherence to stringent guidelines established by D.Lgs. 26/2014.

To address these challenges while upholding principles of animal welfare, there is a growing emphasis on identifying invertebrates as alternative experimental models. Many invertebrate taxa exhibit morphological or functional traits that make them suitable for such purposes. Additionally, adherence to animal welfare standards is ensured through the development of tailored sedation protocols for different invertebrate groups [5].

Molluscs, with their diverse mucous secretions [6-8] provide a compelling model for investigating the molecular, histochemical, and lectin-based characterization of glycoconjugates. This review discusses the potential of molluscs in mucomic research, emphasizing histochemical and lectin-based studies as exemplified by recent research on various species.

Molluscs promising experimental models for the study of mucomics. These organisms produce a variety of glycans in their mucus, which play crucial roles in processes such as locomotion, immune defense, and adhesion to surfaces [9-11]. The study applies an integrated “mucomic” approach, combining proteomics, glycomics, and materials science to understand the relationships between mucus structure and function.

Within the diverse phylum Mollusca, three classes of notable interest - both commercial and scientific - play a key role in animal experimentation and environment monitoring [12]. Among the gastropods, terrestrial species such as *Cornu aspersum* [6] and *Eobania vermiculata* [13] stand out, while marine Gastropoda include Aplysiida [14] and Patellida [15] Order. In freshwater, a gastropod like *Lymnaea stagnalis* [16] serves as an example. These species produce significant amounts of mucus, which not only aids in locomotion and substrate adhesion but also serves functions such as immune defense and osmoregulation [17]. Due to their substantial mucus production, combined with their ease of maintenance and reproduction under laboratory conditions, these gastropods are excellent candidates for research applications.

Regarding bivalves, families such as Mytilidae, Ostreidae, and Veneridae are widely used as environmental bioindicators [18-21] due to their sensitivity to chemical changes in water, exemplified by programs like Mussel Watch [22, 23]. In these species, mucus is produced primarily for feeding purposes and to protect their gills - key filtering organs - from organic and inorganic particulates that could cause mechanical damage; additionally, mucus plays a role in defense against pathogens and environmental contaminants [17, 24-26].

While there are studies on the histochemistry of their mucus [27], using these species in basic research and experimentation entails navigating ethical and legislative challenges in some regions, owing to their complex biology and behavior. In these countries it is easier to obtain permits for research on their paralarvae [28].

## 2. The Glycosylation of Mucins in Molluscs: Structures, Functions, and Environmental Impacts

Mucins are high molecular weight epithelial glycoproteins with a high content of O-linked oligosaccharides, synthesized, stored, and secreted by epithelial mucosal cells, particularly goblet cells, and are characterized by a protein backbone with a variable number of repeated amino acid sequences [29]. These sequences are particularly rich in serine/threonine, which are the attachment sites for O-glycans. There are two main classes of mucins: secreted mucins, which are components of mucus, and membrane-bound mucins. The evolutionary role of mucins in molluscs also highlights how these proteins have adapted to different ecological niches and environmental challenges over time [30]. The synthesis of mucin apoproteins is encoded by a series of genes which in humans are

referred to as MUC (MUC1, MUC2, etc.), and have homologues in many other species. [31]. The mucin apoprotein undergoes significant post-translational modifications, particularly extensive glycosylation. The carbohydrate content represents a notable percentage, ranging from 50% to 90% of the total molecular weight. O-linked oligosaccharide chains prevail, but N-linked chains are also present at the terminal regions, and although they are quantitatively less represented, they are functionally very important. N-linked oligosaccharides tend to be complex and branched. The first sugar in the chain is N-acetylglucosamine, which is linked to an asparagine in the polypeptide chain, part of a specific consensus sequence (Asn-Xaa-Ser/Thr)[6]. Other sugars are added to the initial GlcNAc until a complex branched chain is formed. N-glycosylation begins in the rough endoplasmic reticulum and is completed in the Golgi apparatus. All N-glycans contain a common pentasaccharide core, Man3GlcNAc2. Additional glycosidic residues are added to the pentasaccharide core in the Golgi. O-linked oligosaccharide chains generally contain an initial residue of GalNAc. There are at least eight different types of cores. They are typically less branched than N-linked oligosaccharides. O-glycosylation begins in the Golgi, and sugars are added one by one to the chain through the action of specific glycosyltransferases as the glycoproteins migrate from the Cis to the Trans Golgi [32]. This results in the high heterogeneity of the oligosaccharide chains. Mucins are highly glycosylated proteins that constitute key components of the mucus secreted by molluscan epithelia. They play various roles, including filter feeding, digestion, respiration, maintenance of the mantle cavity, defense against predators and pathogens, adhesion or penetration into substrates, osmoregulation, biomineralization, and signaling through luminescence both within and between species [9, 10, 17, 33, 34]. In addition to their various roles, mucins also influence interactions with aquatic microbiota, contributing to the overall health of molluscs and the stability of the ecosystem. Additionally, mucins found in pseudofaeces contribute to the particulate organic matter present in coastal ecosystems [17]. In mucin structures, oligosaccharide chains are typically connected to the protein backbone via  $\alpha$ -O-glycosidic bonds to serine or threonine residues. The variations in glycosylation and carbohydrate composition of these sugar chains have an impact on the viscoelastic, lubricating, and hydration characteristics of mucus, thereby influencing its functionality [35]. Comparative studies on mucins in various invertebrates and vertebrates could provide valuable insights, emphasizing both the similarities and differences in glycosylation patterns and functional roles. Despite their significance, research regarding the composition, distribution, and variability of mucins in molluscan secretions remains limited. Classical histochemical analyses suggest that the oligosaccharide chains in mucus can exhibit neutral or acidic properties, which result from the presence of carboxylated or sulfated residues [7, 15, 33, 36-39].

However, in situ investigations on the composition of these oligosaccharide chains are even scarcer, indicating the presence of mannosylated, glycosaminoglycan-modified, galactosylated/galactosaminylated, and fucosylated residues, while the presence of sialic acid remains a matter of debate [7, 15, 36-40]. It has been noted that glycosylation varies across different types of secreting cells distributed in various anatomical regions of bivalves, including the mantle, foot, gills, and digestive gland [7, 8, 26, 33, 41, 42]. Additionally, certain molluscan classes, such as gastropods and cephalopods, show variations in mucin composition within the same cell type due to physical factors (hydrodynamics, salinity, temperature, humidity) or specific biological activities (reproduction, growth, locomotion, feeding) [15, 28, 44]. Furthermore, changes in the qualitative and quantitative aspects of mucins have been linked to pollutants, indicating that such variations could serve as biomarkers[43, 45]. Moreover, understanding how environmental changes, such as ocean acidification and global warming, impact mucin glycosylation and composition can help assess the overall health of mollusk populations. As filter-feeding organisms capable of filtering contaminants from the environment, bivalves play a vital role in water quality monitoring [46-49], and they are increasingly regarded as models for human health studies [50]. It is crucial to underscore the need for further research to explore the variability and function of mucins in various developmental stages of molluscs and across different species, as significant gaps remain in the current literature. Understanding the complexity of mucin glycosylation in molluscs not only provides fundamental insights into their biology but also contributes to environmental monitoring strategies and potential biomedical applications.



### 3. The Role of Mucins in Molluscs: Immune Protection, Digestion, and Environmental Adaptations

Mucomics, the study of mucins and their composition, provides an important window into the physiological processes in molluscs, particularly concerning immunity and digestion [6]. Mucins are highly glycosylated proteins that, as key components of mucus, perform multifunctional roles vital for the survival of these aquatic organisms. One of the main functions of mucins is their ability to act as physical barriers [51]. They protect molluscs from pathogens, pollutants, and other harmful agents present in the aquatic environment. Mucins create a protective layer that not only facilitates immune defense but also provides a means for the physical removal of foreign particles. Through adhesion and interaction mechanisms, mucins can trap and neutralize pathogens, preventing infections and diseases. This function is particularly important given the continuous exposure of molluscs to microorganisms and pollutants [52]. Furthermore, mucins play a significant role in modulating immune responses. The varied composition of mucins in response to environmental stimuli highlights how these organisms can adapt their defenses according to specific challenges [53]. For example, the group of mucins produced may change when exposed to new pathogens, suggesting a dynamic adaptation mechanism and an ability to activate the immune response in molluscs. From a digestive perspective, mucins are essential for lubricating food, facilitating the formation of a more manageable food bolus, and ensuring smooth passage through the digestive system. Due to their viscoelastic properties, mucins make food easier to handle and process, thereby improving the efficiency of the digestive process. Variations in mucin composition can also reflect the specific diet of molluscs, revealing how these animals adapt to different nutritional sources, from plant materials to plankton. Analyzing mucins and their variations also provides important insights into the health of molluscan ecotypes and environmental changes [54]. Mucins show significant diversity in relation to salinity, temperature, and other environmental conditions. These variations can serve as biomarkers to indicate environmental health and water quality, providing a useful method for monitoring the effects of pollution and environmental changes on biodiversity [55]. Recent studies have particularly highlighted the importance of mucin glycosylation, which can significantly influence the rheological properties of mucus [52]. Variations in glycosylation can have direct impacts on the lubricating and adhesive properties of mucus, crucial for the survival and functionality of molluscs in variable environments [56]. Understanding how different species of molluscs manipulate their mucins can also provide insights into addressing ecological and health-related challenges [57]. Finally, delving into the mechanisms of mucins in molluscs has not only ecological relevance but can also offer avenues for applications in medicine and biotechnology. Mucins could be harnessed for their potential in biomolecular contexts, opening new paths for research in human health and veterinary medicine. In summary, mucomics represents a promising field for uncovering the complexities of physiological processes in molluscs, contributing to a deeper understanding of their biology, ecology, and importance in aquatic ecosystems. Studying how mucins influence immunity, and digestion can reveal vital information for both species conservation and advancements in biomedical technologies.

## 4. Models Study

### 4.1. Gastropods

**Terrestrial Gastropods** - Helicidae such as the garden snail *Cornu aspersum* and the chocolate band snail *Eobania vermiculata* are prominent terrestrial gastropods extensively studied for their responses to environmental stressors, particularly aluminum exposure. Research on *Cornu aspersum* identified three types of mucus—protective, adhesive, and lubricating—each serving distinct mechanical and functional roles. Analytical approaches such as proteomic, glycomic, and biophysical methods, including advanced mass spectrometry and rheological assays, have characterized its macromolecular composition. These studies highlighted the high viscosity and elasticity of adhesive mucus compared to the simpler lubricating mucus, demonstrating its ecological significance [6].

For *Eobania vermiculata*, histochemical and lectin-histochemical techniques have been employed to analyze the effects of aluminum exposure on mucin secretion. Methods like PAS and Alcian Blue

staining revealed a reduction in glycosylation and the production of acidic glycans, impairing the viscosity and protective properties of mucus. Specific lectins, such as UEA I and WGA, detected fucosylated and GlcNAc-linked glycans, respectively, emphasizing the impact of environmental stressors on mucosal functionality [58, 59]. These findings underscore the vulnerability of terrestrial gastropods to contaminants and their potential as models for studying the impact of pollutants on mucin structure and function.

**Freshwater Gastropods** - Freshwater species, such as the great pond snail (*Lymnaea stagnalis*), are key models for monitoring heavy metal pollution in aquatic environments. *Lymnaea stagnalis* has demonstrated a high capacity for bioaccumulating cadmium (Cd) and zinc (Zn), reflecting site-specific contamination levels. This species is particularly valuable for assessing pollution dynamics in freshwater ecosystems, as its ability to sequester metals through mucus and other tissues provides critical insights into the effects of contaminants on molluscan physiology.

Other freshwater gastropods, such as river snails *Viviparus spp.*, exhibit a dual feeding strategy—deposit-feeding and suspension-feeding—that allows them to reflect sediment-associated metal concentrations. This feeding behavior enhances their utility as bioindicators for sediment-bound pollutants, offering a comprehensive understanding of contamination in aquatic systems [60].

**Marine gastropods** - including veined rapa whelk *Rapana venosa* and the moon snail *Neverita didyma*, exhibit significant bioaccumulation of Cd in coastal ecosystems. These species have been studied for their capacity to assess pollution levels in marine environments, particularly heavy metals. The high bioaccumulation potential of *Rapana venosa* makes it a valuable sentinel species for tracking cadmium contamination. Similarly, *Neverita didyma* has been employed to monitor cadmium levels, emphasizing the role of marine gastropods in evaluating the ecological impact of heavy metal pollution [60].

In the Mediterranean region, gastropods such as *Phorcus turbinatus* and *Patella caerulea* have been used to monitor copper (Cu) and Zn pollution due to their sensitivity to these metals. These species provide essential data on coastal metal contamination, contributing to efforts to manage and mitigate pollution in marine ecosystems [61].

#### 4.2. Bivalves

Bivalves are a diverse group of molluscs characterized by their filter-feeding ability, which enables them to extract nutrients from suspended particles in the water. This feeding behavior also makes them effective bioindicators of environmental contamination, as they accumulate pollutants from their surroundings in their tissues and mucus. Across taxa, heavy metals such as Cd, lead (Pb), nickel (Ni), Cu, and Zn are the most commonly studied contaminants. The mucus, enriched with glycans and proteins, serves a dual role as a protective barrier and a medium for trapping contaminants. For example, *Ruditapes philippinarum* efficiently traps organic pollutants like polycyclic aromatic hydrocarbons (PAHs), although prolonged exposure can cause oxidative stress, lipid peroxidation, and DNA damage [62, 63].

Internally, the digestive glands and gills of bivalves act as primary sites for metal sequestration, with lysosomes in digestive cells playing a pivotal role in detoxification. These processes underscore the importance of molluscs as bioindicators for environmental contamination, providing valuable insights into pollutant dynamics and their ecological impact [64, 65]. The mucus of bivalves plays critical roles in particle transport, pathogen defense, and environmental interactions. Its composition, enriched with glycans and proteins, varies under different stressors, reflecting changes in functionality.

Techniques such as histochemical staining and biochemical assays have provided insights into how environmental pollutants alter mucus composition and secretion.

**Marine bivalves** - including *Mytilus galloprovincialis*, *Chamelea gallina*, and *Ruditapes philippinarum*, are extensively studied for their responses to environmental contaminants.

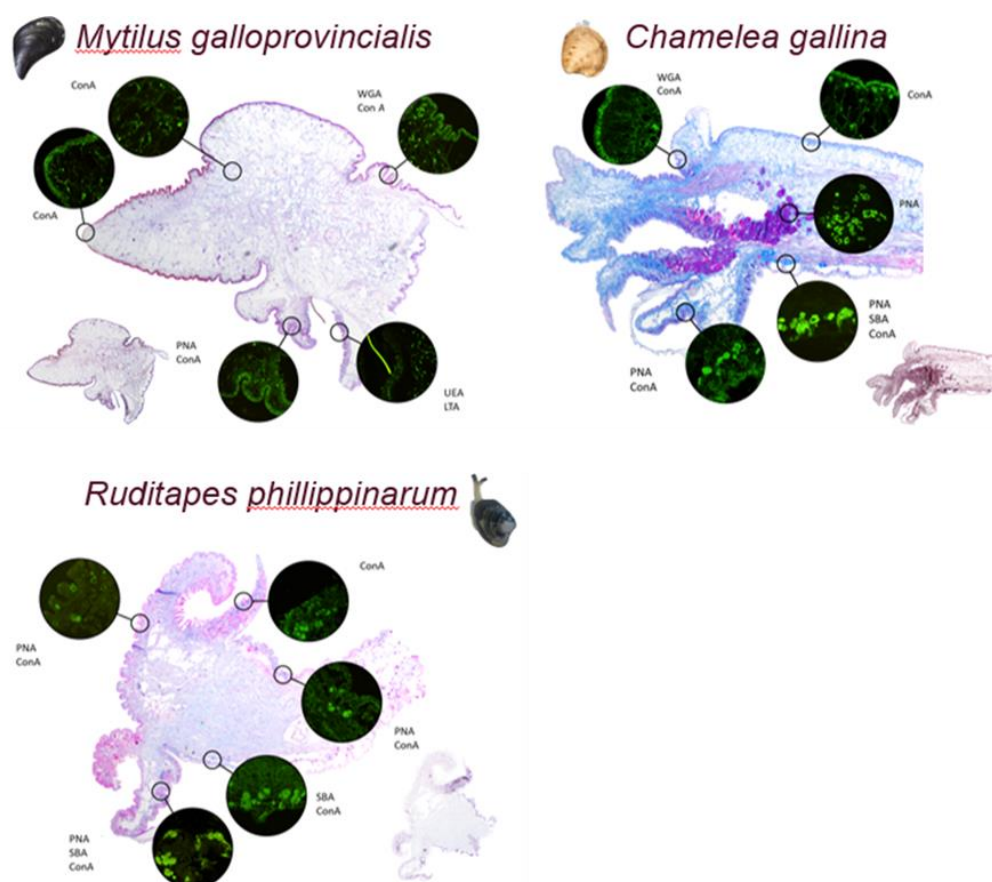
An example of the difference in their tissues and the different responses to histochemical treatments, which we will discuss later, can be seen in Figure 1.

The Mediterranean mussel *Mytilus galloprovincialis* is a recognized bioindicator and is widely used for biomonitoring programs such as "Mussel Watch." Its sessile nature and high

bioaccumulation capacity make it ideal for studying heavy metals, including arsenic (As), Pb, chromium (Cr), manganese (Mn), mercury (Hg), and Zn [64, 66, 67]. Similarly, the brown mussel *Perna perna* and oysters such as *Crassostrea angulata* and *Crassostrea virginica* have been employed in laboratory and field studies to evaluate the uptake, loss, and biological effects of these metals. In studies along the Chinese Bohai Sea, *Crassostrea talienwhanensis* was found to have a particularly high capacity for bioaccumulation of Cu and Zn compared to other species, while *Rapana venosa* showed significant cadmium bioaccumulation [63, 68]. Notably, the Manila clam *Ruditapes philippinarum* exhibited the highest Ni content among studied species, emphasizing its utility in assessing Ni contamination [63]. Advanced techniques such as immunohistochemistry, in situ hybridization, and lectin arrays have been used to map gene expression and glycosylation patterns, while ICP-AES quantified seasonal bioaccumulation of trace metals, distinguishing anthropogenic from natural sources [69]. Studies have also demonstrated the role of *Mytilus galloprovincialis* mucus in mitigating metal toxicity, particularly for Zn and iron (Fe), underscoring its ecological relevance [70].

The Venus striped clam *Chamelea gallina* has been studied in normal condition [71] and for pollutant-induced alterations in mucin secretion. Histochemical and lectin histochemical analyses, revealed reductions in glycan diversity, acidity, and viscosity under aluminum exposure. These changes impair the protective and pathogen-defense functions of mucus, highlighting the vulnerability of *Chamelea gallina* to aluminum (Al) pollutants [18].

*Ruditapes philippinarum* has shown a significant capacity for bioaccumulating polycyclic aromatic hydrocarbons (PAHs) and heavy metals like Cd and Ni. Biochemical assays and molecular markers, including oxidative stress indicators like DNA strand breaks and lipid peroxidation, have been used to detail the impacts of these contaminants. High-performance liquid chromatography (HPLC) has been instrumental in elucidating bioaccumulation pathways and tissue-specific detoxification mechanisms [63, 72]. *Ruditapes philippinarum* has recently also been tested for contamination by rare earth elements such as Lanthanum (La) [58]



**Figure 1.** Representative different morphology and responses to histological and histochemical staining in the mantle of various marine bivalves. In green on a dark field, the fluorescence response of the mucus-secreting cells present in the different folds of the mantle to the different lectins can be observed.

**Freshwater bivalves** - such as *Dreissena polymorpha* (zebra mussel) and *Pletholophus swinhoei*, play a crucial role in pollution monitoring in riverine and lacustrine ecosystems.

The first has been widely used to assess trace metal pollution in freshwater systems like the Po River in Italy. Studies revealed increasing concentrations of Cd, Pb, Cr, and Ni in mussel tissues over time, reflecting the intensification of pollution in aquatic ecosystems. Stable Cu and Zn levels, as well as consistently low Hg levels, highlight the potential of *Dreissena polymorpha* as a reliable bioindicator for specific contaminants [65].

*Pletholophus swinhoei* has focused on site-specific variations in heavy metal concentrations, including As, Ba, Cr, and Ni. These findings provide valuable data for evaluating localized pollution impacts and guiding environmental management strategies [65].

In bivalves, *Chamelea gallina* mucus [18], serves a critical role in particle transport and pathogen defense. Using Alcian Blue staining and lectins like SBA and PNA, researchers identified significant reductions in glycan diversity and acidity under aluminum exposure, leading to functional impairments. *Mytilus galloprovincialis*, a widely studied bioindicator, employs mucus to trap and detoxify metals such as zinc and iron. Studies by Miglioli et al. (2024) [70] utilized immunohistochemistry, in situ hybridization, and lectin arrays to map gene expression and glycosylation patterns. Seasonal bioaccumulation of trace metals was quantified through ICP-AES, demonstrating the ecological role of mucus in mitigating metal toxicity. *Ruditapes philippinarum*, as studied by Li et al. (2020) [72], uses mucus to trap polycyclic aromatic hydrocarbons (PAHs). Histological and biochemical analyses, including PAS staining and oxidative stress biomarkers such as DNA strand breaks and lipid peroxidation, revealed that mucus entraps contaminants but is affected by oxidative damage due to PAH exposure.

The functional properties of molluscan mucus extend beyond the individual organism, influencing ecosystem dynamics. According to Davies & Hawkins (1998) [17], mucus production can consume up to 70% of a mollusk’s energy budget, underscoring its ecological importance. Mucus contributes to organic particulate matter, supports locomotion, and forms barriers against environmental threats. However, as highlighted across these studies, environmental pollutants like aluminum and PAHs can disrupt mucus composition and functionality, with significant implications for molluscan health and ecosystem stability. The integration of advanced techniques, including mass spectrometry, histochemical staining, and lectin arrays, has been instrumental in elucidating the complex structure-function relationships of mucus and its response to environmental stressors.

5. Investigative Techniques and Contaminants

Across molluscs, a range of investigative techniques has been applied to study mucus composition and pollutant bioaccumulation. Proteomic and glycomic analyses, including mass spectrometry and glycan profiling, have been essential in characterizing mucus properties in terrestrial species. Histochemical staining techniques, such as PAS and Alcian Blue and High Iron Diamine, combined with lectin-histochemical methods, have been widely used to investigate mucin glycosylation patterns and their alterations under environmental stressors like aluminum. Lectin widely used to perform these studies are present in Tab. 1.

For aquatic gastropods, biochemical assays and tissue analysis methods have been employed to assess bioaccumulated heavy metals, including Cd, Zn, and Cu. These studies utilize advanced techniques such as HPLC and ICP-AES to quantify contaminant levels and evaluate their effects on molluscan health and environmental interactions.

**Table 1.** - Lectins employed with their diluting buffers, working dilutions, and inhibiting sugars. Binding specificities: **PNA:** Galβ1,3GalNAc; **SBA:** GalNAc; **WGA:** (GlcNAcβ1,4)n; **LTA:** L-Fuca1,6GlcNAc; L-Fuca1,2Galβ1,4(L-Fuc1,3)GlcNAcβ1,6R; **UEA I:** Fuca1,2; **AAL:** Fuca1,6GlcNAc-βNAsn; Fuca1,3,Fuca1,4; **SNA:** Neu5Aca2,6Gal/GalNAc; **MAA II:** Neu5Aca2,3Galβ1,4GlcNAc; **ConA:** D-Man, D-Glc.

| Lectin | Origin           | Buffer | Dilution | Inhibitory Sugar Conc. |
|--------|------------------|--------|----------|------------------------|
| PNA    | Arachis hypogaea | Hepes  | 10 mg/mL | 0.2 M Gal              |
| SBA    | Glycine max      | Hepes  | 20 mg/mL | 0.2 M GalNAc           |



|               |                                 |       |          |                    |
|---------------|---------------------------------|-------|----------|--------------------|
| <b>WGA</b>    | <i>Triticum vulgare</i>         | Hepes | 20 mg/mL | 0.5 M GlcNAc       |
| <b>LTA</b>    | <i>Tetragonolobus purpureus</i> | Hepes | 20 mg/mL | 0.2 M L-Fuc        |
| <b>UEA I</b>  | <i>Ulex europaeus</i>           | Hepes | 10 mg/mL | 0.2 M L-Fuc        |
| <b>AAL</b>    | <i>Aleuria aurantia</i>         | Hepes | 10 mg/mL | 0.2 M L-Fuc        |
| <b>SNA</b>    | <i>Sambucus nigra</i>           | Hepes | 20 mg/mL | 0.2 M Neu5Ac       |
| <b>MAA II</b> | <i>Maackia amurensis</i>        | Hepes | 20 mg/mL | 0.2 M Neu5Ac       |
| <b>ConA</b>   | <i>Canavalia ensiformis</i>     | Hepes | 20 mg/mL | 0.1 M M $\alpha$ M |

## 6. Contaminants and Mucus Functionality

Across taxa, heavy metals such as Cd, Pb, Ni, Cu, and Zn are the most commonly studied contaminants. Gastropods like *Rapana venosa* and bivalves such as *Mytilus galloprovincialis* exhibit significant alterations in mucus production and composition when exposed to these pollutants. The mucus, enriched with glycans and proteins, serves a dual role as a protective barrier and a medium for trapping contaminants. For example, *Ruditapes philippinarum* efficiently traps organic pollutants like polycyclic aromatic hydrocarbons (PAHs), although prolonged exposure can cause oxidative stress, lipid peroxidation, and DNA damage [62, 63].

Internally, the digestive glands and gills of bivalves act as primary sites for metal sequestration, with lysosomes in digestive cells playing a pivotal role in detoxification. These processes underscore the importance of molluscs as bioindicators for environmental contamination, providing valuable insights into pollutant dynamics and their ecological impact [64, 65].

## 7. Conclusions

Molluscs are excellent environmental indicators, as demonstrated by the use of many bivalves in international monitoring programs. A mucomic approach to the study of their mucus provides important information on its ecological, physiological and structural significance. Advances in histochemical, proteomic and glycomic techniques have allowed a more detailed characterization of mucins, shedding light on their physiological functions and responses to environmental stresses. However, significant gaps remain in our understanding of the variability of mucins among species, life stages and environmental conditions. Further research is needed to explore these variations, their ecological implications and potential biomedical applications, or as sentinels, even for human health. By integrating molecular and biochemical studies, future investigations could improve our understanding of the structure-function relationships of mucins and their role in environmental monitoring.

**Author Contributions:** For research articles with several authors, a short paragraph specifying their individual contributions must be provided. Conceptualization, D.M., M.M. and M.V.G.; writing—original draft preparation, D.M., D.S., M.V.G.; writing—review and editing, C.D.B., D.S., M.M.; supervision, D.M., M.M., M.V.G. All authors have read and agreed to the published version of the manuscript.

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