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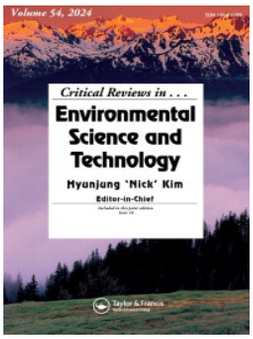

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Biosafety of two-dimensional molybdenum disulfide nanomaterials: Natural and engineered transformation pathways and their role in the toxicity profile

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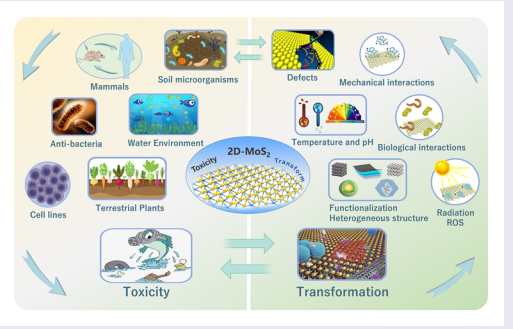
ABSTRACT



As a rising star in the post-graphene era, two-dimensional molybdenum disulfide nanomaterials (2D-MoS_2) are taking the scientific community by storm due to their fascinating physicochemical properties. An accurate and comprehensive understanding of the biosafety of 2D-MoS_2 is urgently needed before further translating its promising performance in laboratory scale studies into actual applications in practice. The toxicity of 2D-MoS_2 is highly correlated with their specific parameters, which means that artificial modifications or natural aging scenarios of 2D-MoS_2 should be included in the corresponding toxicological reports. In this review, we first critically review the toxicological data on 2D-MoS_2 obtained in various *in vitro* and *in vivo* toxicity evaluation models. Subsequently, we summarize the different approaches of modification and natural aging pathways of 2D-MoS_2, including size and layer structure changes, phase transformation, surface defects, bio-molecular hybridization, surface functionalization, etc. We further critically discuss how these transformations will affect the toxicity and mechanisms of interaction of 2D-MoS_2 with organisms, emphasizing the necessity of adopting interdisciplinary approaches to fully reveal the correlation between 2D-MoS_2 transformations and toxicity profile. Based on the critical review of existing studies, we identify current knowledge gaps and priorities for future research, highlighting the necessity of establishing exposure protocols with uniform criteria, developing precautionary toxicity mitigation strategies and predictive models, and incorporating transformation scenarios throughout the toxicity assessment procedures of 2D-MoS_2.

KEYWORDS Molecular mechanisms; MoS_2 nanosheet; transformation; toxicity

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1. Introduction

Two-dimensional transition metal dihalides (TMDs) have emerged as one of the most promising inorganic nanomaterials (Chen et al., 2021; Li et al., 2017). Among them, the emerging two-dimensional molybdenum disulfide nanomaterials (2D-MoS_2) have attracted great research interest and application enthusiasm due to their excellent optoelectronic and catalytic properties (Radisavljevic et al., 2011; Wang et al., 2015a; Yin et al., 2014; Zhang et al., 2019), and are developing into a novel nano-platform (Cao et al., 2021a). As with most engineered nanomaterials (ENMs), 2D-MoS_2 will inevitably be released into the environment during the life cycle of development, use, and disposal (Zeng et al., 2022). Although global consumption has not been reported, the large-scale production and rapid commercialization of 2D-MoS_2 have gradually raised concerns regarding their biosafety (Huang et al., 2014; Xu et al., 2020b). Compared to microplastics or other traditional 3D ENMs (CuO, TiO_2, ZnO, etc.), 2D-MoS_2 may have completely different adverse outcome pathways and mechanisms due to their unique physicochemical properties and nanosheet structure. To date, the health and environmental risk of 2D-MoS_2 is still an emerging topic, and many unknown questions are waiting to be answered. A clustering analysis of literatures on 2D-MoS_2 in the Web of Science (Figure 1) shows that reports on their toxicity have only become available after 2014. In 2015–2016, studies on the cytotoxicity of 2D-MoS_2 began to emerge. Since then, the number of toxicity studies on 2D-MoS_2 has increased yearly (bar chart in Figure 1) and is expected to keep increasing in the future. From 2020 onwards, different properties of 2D-MoS_2, including mesoporosity, environmental fate, chemical stability, and layer numbers, have gradually become important considerations in the toxicity evaluation of these materials (Figure 1).

Due to the diversity of physicochemical properties, 2D-MoS_2 are sensitive to external factors (Cao et al., 2021a). Therefore, 2D-MoS_2 are easily transformed either by engineered modification or by environmental/biological aging processes (e.g., UV, visible light, oxidation, biological interactions, etc.) (Celina et al., 2019; Fan et al., 2018; Lee et al., 2019; Wang et al., 2016b). The transformation includes, but is not limited to, changes in the physical structure of the nanosheets (e.g., nanopores, size, lattice structure, etc.) and their chemical properties (e.g., atom vacancies, dangling bonds, etc.). These transformations may further lead to changes in the environmental behavior of 2D-MoS_2, such as agglomeration and chemical dissolution, which have been shown to closely affect their environmental risk (Qiu et al., 2017; Zhi et al., 2017).

The existing relevant reviews only focus on the progress of 2D-MoS_2 synthesis methods and their applications in environmental, electrochemical, biomedical, and energy storage fields (Gan et al., 2017; Ganatra & Zhang, 2014; Vilian et al., 2019; Wang & Mi, 2017; Yadav et al., 2019), but few reviews have critically evaluated their toxicity study progress. Therefore, a more extensive analysis is urgently needed of the transformation pathways, environmental behavior, toxicity profiles of 2D-MoS_2, and their interrelationships. Such an analysis will help identifying and controlling the health and environmental risks of 2D-MoS_2 and guide their safe design and sustainable use. This review aims to (a) systematically review the current knowledge of 2D-MoS_2 toxicity to *in vitro* cell lines, mammals, aquatic organisms, plants, and microorganisms; (b) summarize in detail the engineered modification pathways and natural aging factors of 2D-MoS_2 and to establish connections between their transformation and physicochemical properties; (c) critically discuss the role of 2D-MoS_2 transformation in its toxicity and risk profiles; (d) identify current knowledge gaps and future research priorities.

2. Toxicity of two-dimensional molybdenum disulfide nanomaterials

2.1. In vitro *toxicity of 2D-MoS_2 to cell lines*

Currently, 2D-MoS_2 are showing great potential for applications of biosensors and drug delivery. Therefore, it is an important task to evaluate the toxicity of 2D-MoS_2 at the cellular and

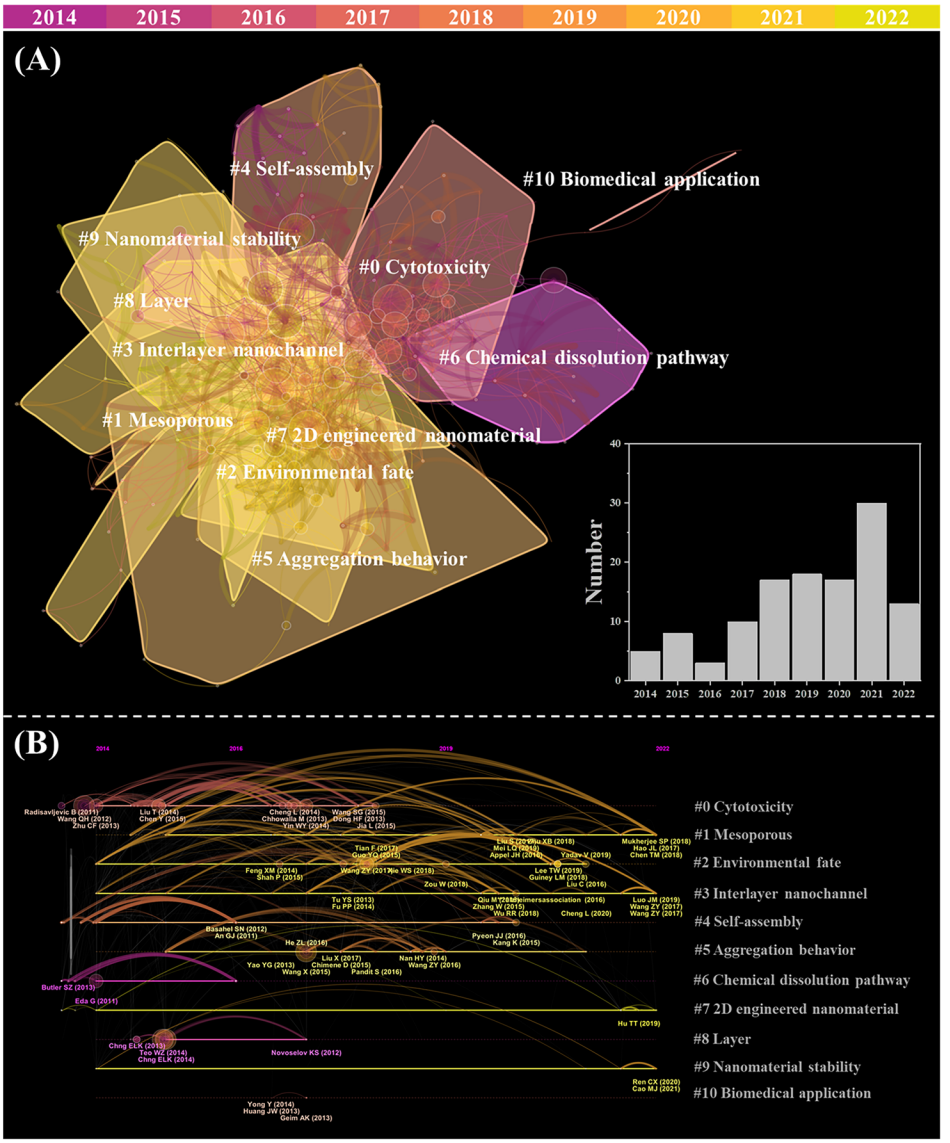

Figure 1. Current trends and evolution of the research based on the literature matching "2D-MoS_2/MoS_2 nanosheet", "toxicity" and "risk" in the Web of Science database (statistics up to 2022), as determined by citespace (V5.6.3) software. Each round node denotes a single retrieved article, and the node's size positively correlates with the frequency of the corresponding article being cited. The lines between the nodes represent a correlation between the two studies. The thicker the line, the stronger the correlation. The shaded areas with different colors indicate the clustering results by topic. The smaller the value of the topic's number (#no), the more keywords are included in the corresponding cluster.

subcellular levels to ensure their biosafety in biomedical applications. To date, it is generally accepted that the exfoliated 2D-MoS_2 have low cytotoxicity, especially when compared to its corresponding bulk form or graphene-based 2D nanomaterials. For example, no significant decrease in cell viability was observed in several cell models, including rat pheochromocytoma cells (PC12), adrenal medulla endothelial cells (RAMEC), human monocyte-derived leukemia cells (THP-1), human lung carcinoma epithelial cells (A549), human Gastric cancer cells (AGS), human epithelial kidney cells (HEK293f), and human adipose-derived stem cells (MSCs), upon exposure to exfoliated 2D-MoS_2 at a wide range of exposure concentrations, even when considering nanosheet synthesis pathways and exposure time difference (see Table S1 for details)

(Appel et al., 2016; Corazzari et al., 2014; Ghim et al., 2018; Kaur et al., 2018; Moore et al., 2017; Rashkow et al., 2015; Shah et al., 2015; Teo et al., 2014). Based on these results, researchers consistently claim that the nanosheets exfoliated from bulk-MoS_2 possess excellent biocompatibility. However, we believe that more caution should be taken in drawing such conclusions, as these results are only based on simple quantification of cell viability and lack evidence of cell morphology or monitoring at metabolic and transcriptional levels. Importantly, contradicting results have been reported demonstrating non-negligible cytotoxicity of 2D-MoS_2. For example, 30 μg/mL of 2D-MoS_2 significantly induced a decrease in cell viability of human hepatoma HepG2 cells (Liu et al., 2017). In this case, elevated levels of reactive oxygen species (ROS), loss of mitochondrial membrane potential (MMP), and membrane damage were also detected even at relatively low exposure concentrations (<8 μg/mL). This is in contrast to several previously reported studies, none of which observed the release of lactate dehydrogenase, a marker of membrane damage (Shah et al., 2015; Sobańska et al., 2020; Teo et al., 2014; Xie et al., 2022).

We have carefully analyzed these contradictory findings. Overall, inconsistent experimental designs and material preparation processes make it difficult to compare the available experimental results. Firstly, differences in exposure concentration will lead to large fluctuations in cytotoxicity. Teo et al. (2014) reported that 0–200 μg/mL of 2D-MoS_2 exposure for 24 h caused no significant changes in the viability of A549 cell lines (~81%–97% of the control), while cell viability decreased to ~50% of the control group at 400 μg/mL exposure. Secondly, the cell culture system plays a key role in the outcome of cytotoxicity tests on 2D-MoS_2. For instance, Sobańska et al. (2020) compared the internalization of 2D-MoS_2 by HepG2 cells in a conventional monolayer (2D) and a 3D spherical culture system (more closely resembles the tissue or tumor morphology). They showed that HepG2 cells grown in the conventional 2D culture system internalized 2D-MoS_2 (250 μg/mL) less efficiently, but 2D-MoS_2 were effectively internalized by the outer layer of cells in the 3D spherical culture system. Also, the type of cells may affect cytotoxicity. Kaur et al. (2018) reported that exposure to 2D-MoS_2 dispersion (10 μg/mL and 14 μg/mL) caused only negligible mechanical damage to the normal human keratinocytes HaCaT cells, but induced significant loss of cell viability in two tumor cell lines (human breast cancer MCF7 cells and acute myeloid leukemia U937 cells) in the same batches tested. This representative case, along with other available results, strongly suggests that the selection of different cell lines and exposure conditions may lead to distinctly different toxicity test results. Besides the species tested and exposure system, the nanosheet layer structure and lateral dimensions have also been reported to affect their cytotoxicity (Chng et al., 2014; Kaur et al., 2018; Moore et al., 2017; Zhou et al., 2019b). Therefore, a universal judgment on the cytotoxicity of 2D-MoS_2 cannot be derived from any single result.

Despite the large number of studies focusing on the cytotoxicity of 2D-MoS_2, the mechanism of action was only superficially explored. These toxic effects can be readily summarized as oxidative stress or internalization-mediated cell viability changes, with a tested dose range of 0.001–400 μg/mL (Table S1). As an extension of these known toxicity mechanisms, Xu et al. (2020a) revealed a novel mechanism of 2D-MoS_2-induced human bronchial epithelial cells (BEAS-2B) and THP-1 cells death. Briefly, unsaturated metal atoms on the 2D-MoS_2 surface may cause lysosomal membrane damage *via* phospholipid deprivation and oxidative stress pathways, which in turn trigger the intracellular leakage of Fe^{2+}. Subsequently, Fe^{2+} activates the intracellular Fenton reaction, causing ROS accumulation, lipid peroxidation, and loss of membrane stability, and ultimately cell death (Xu et al., 2020a). The mechanism by which 2D-MoS_2 triggers cellular autophagy through interfering with the cell surface amyloid precursor proteins and activating the mechanistic target of rapamycin (mTOR) signaling pathway has also been reported recently (Zhou et al., 2019b). However, there are still several questions regarding the cytotoxicity mechanism of 2D-MoS_2. For example, the cellular internalization pathway and the ultimate intracellular fate of 2D-MoS_2 are still poorly understood. The available evidence suggests that 2D-MoS_2 follow similar cellular uptake patterns as other nanoparticles such as graphene derivatives and Ag nanoparticles (Tu et al., 2018), which has been thoroughly investigated for aquatic

single-celled microalgae (Zeng et al., 2022). Considering the inevitable differences due to the presence of algal cell walls, the use of specific pharmacological inhibitors coupled with higher-resolution bio-imaging techniques is recommended to accurately differentiate the cell internalization mechanisms.

In summary, although it has been studied for several years, it is premature to discuss the further application of 2D-MoS_2, especially in the biomedical field, until its interactions with cells are fully elucidated. To completely resolve the biosafety issues of 2D-MoS_2, we strongly recommend screening sensitive and appropriate exposure models on different test species, with the aim to establish uniform test procedures to obtain comparable toxicity data.

2.2. *In vivo* toxicity of 2D-MoS_2 to mammals

The toxicity of xenobiotics is closely related to the overall coordinated response of the organism; therefore, based on the findings of *in vitro* experiments, the organ accumulation, inflammation, and immune response of 2D-MoS_2 should be further elucidated *in vivo*. In a representative study, acute (40 h) oropharyngeal aspiration of bulk-MoS_2 (2 mg/kg mice weight) resulted in partial inflammation in the lungs of mice, while no inflammatory response was observed upon sub-chronic exposure (21 days). Compared to bulk-MoS_2, 40 h or 21 days of oropharyngeal exposure of exfoliated 2D-MoS_2 at the same dose (2 mg/kg mice weight) had negligible toxicity to mice lungs (Wang et al., 2015c). Another report showed that MoS_2 2D nanodots were bio-transformed *in vivo* (from Mo (IV) to Mo (VI)) upon undergoing transport mediated by protein corona bridges and accumulated mainly in the liver and spleen, donating its Mo for Mo-cofactors biosynthesis (Cao et al., 2021a). There are also several reports on surface-functionalized 2D-MoS_2 (e.g. polyethylene glycol (PEG)-functionalized 2D-MoS_2 and polyvinylpyrrolidone-modified 2D-MoS_2), with no significant *in vivo* toxicity observed in mice through intragastric, intravenous, and intraperitoneal injections at 3.4–100 mg/kg mice weight (Liu et al., 2014; Mei et al., 2019). Recently, 3D *in vitro* cell culture techniques that more closely resemble real tissues are gradually being used in cytotoxicity evaluation. This approach allows researchers to obtain exposure information closer to the *in vivo* environment without using animals. However, this is not a complete substitute for the response of the whole organism. Careful design of *in vivo* experiments based on different potential exposure routes in humans (e.g., inhalation, ingestion, direct exposure, etc.), and different material properties are needed to validate or reconfirm the results of tests using *in vitro* cell lines.

2.3. Toxicity of 2D-MoS_2 to aquatic microalgae and fish

The increasing widespread use of 2D-MoS_2 in water treatment, including membrane separation, heavy metal, and organic pollutant adsorption, will lead to their inevitable release and potential risk to aquatic ecosystems (Wang & Mi, 2017). The aquatic toxicity of 2D-MoS_2 to single-celled microalgae (*Chlorella vulgaris*) has been studied in most detail. The 96-h exposure to 2D-MoS_2 caused a series of adverse effects on *Chlorella vulgaris*, including growth inhibition (72.8% at 25 mg/L), decreased chlorophyll content (34.1% at 25 mg/L), depolarization of MMP (29.5% at 1 mg/L), and significant accumulation of intracellular ROS (37.5% at 1 mg/L) (Tong et al., 2019; Zeng et al., 2022, 2021; Zou et al., 2019, 2018). Growth inhibition, MMP loss, and oxidative stress were significantly alleviated after another 96-h recovery period, but chloroplast damage showed limited recoverability (Zou et al., 2020). Notably, the selection of algae tested and the exposure condition may lead to different toxicity observations. For example, exposure to 0.05 and 0.1 mg/L 2D-MoS_2 significantly promoted the growth of *Dunaliella salina*, whereas 0.1 and 1 mg/L 2D-MoS_2 had no significant effect on *Nostoc sphaeroides* growth (Chen et al., 2021; Luo et al., 2021). This suggests that it is essential to obtain comparable toxicity data through a consistent experimental design. Moreover, there is a disagreement in existing reports regarding

the effects on membrane permeability. Zou et al. (2018) found a significant decrease in membrane permeability of *Chlorella vulgaris* exposed to 0.1–1 mg/L of 2D-MoS_2 in a dose-dependent relationship. In contrast, Zeng et al. (2022) reported 25.8–71.8% increase in the permeability of *Chlorella vulgaris* membranes at the same 2D-MoS_2 concentrations (0.1–1 mg/L). Clearer evidence is needed to clarify this point, such as the selection of membrane permeability markers (e.g., lactate dehydrogenase, etc.) for bidirectional validation.

In addition to single-celled microalgae, Zou et al. (2021b) found adverse effects of 2D-MoS_2 at 1–10 mg/L on *Danio rerio* embryos (2.5 h–7 days after fertilization), including delayed hatching of larvae (80.7–83.2%), reduced heart rate, and increased malformations (manifested as pericardial edema, yolk sac edema, and caudal malformation). However, no data were found on 2D-MoS_2 effects on other aquatic model test organisms, such as widely distributed fish, benthic organisms, crustaceans, and bivalves. Therefore, there is an urgent need to extend the data base on 2D-MoS_2 aquatic toxicity. Given the prevalence of humans feeding on fish and aquatic crustaceans, the concentration of 2D-MoS_2 in the waters of typical culture areas should be surveyed. Meanwhile, based on the existing toxicity data, the potential transfer of 2D-MoS_2 along the aquatic food chain to higher trophic levels and the trophic enrichment or dilution patterns need to be further clarified.

2.4. Toxicity of 2D-MoS_2 to terrestrial plants

Current research on the effects of 2D-MoS_2 on terrestrial plants is mainly in the agricultural field. Zhao et al. (2022) reported that there were no significant changes in rice (*Oryza sativa L.*) after four weeks of growth in soil amended with 2D-MoS_2 (10 and 100 mg/kg) for a range of phenotypic or biochemical endpoints, including biomass, photosynthetic capacity, and oxidative stress. Nano-enabled agriculture based on 2D-MoS_2 has been reported to have beneficial effects on key crops. Contrary to their analogue graphene oxide, the aquaporin gene was activated by 2D-MoS_2, which facilitated the uptake and *in vivo* transport of water in rice (Li et al., 2018; Zhou & Hu, 2017). Li et al. (2023) reported that 2D-MoS_2 enhanced the biological nitrogen fixation and antioxidant capacity of soybean (*Glycine max*) by sustainably releasing Mo and S species and efficiently incorporating them into the synthesis of nitrogenase, molybdenum-based enzymes and thiol-containing antioxidants as cofactors. This was supported by another study showing that 2D-MoS_2 promoted nitrogen assimilation and enzymatic reaction efficiency in *Oryza sativa L.* by providing additional sources of Mo and S (Li et al., 2018). However, the assessment may be influenced by the experimental design, including the species tested and the route of exposure. For example, no uptake and transport of Mo ions by cucumber (*Cucumis sativus*) was reported, which may be related to the different crops selected (Song et al., 2019). Although no adverse effects were observed under soil culture conditions, irreversible (experiencing a 7-day recovery period) decreases in root length (26.3–69.9%) and tip number (22.2–66.0%) were reported in *Oryza sativa L.* hydroponically exposed to 5–20 mg/L of 2D-MoS_2 (Zou et al., 2023). The above laboratory results still need to be further clarified in more realistic exposure scenarios, including comparisons of soil cultivation, hydroponics, and foliar fertilization, as well as thorough validation under field conditions.

2.5. Effects of 2D-MoS_2 on microorganisms

In this section, we review the existing knowledge to summarize the mechanisms of the interaction of 2D-MoS_2 with microorganisms leading to toxic effects as well as the novel contribution of material functionalization to its antimicrobial application.

Soil serves both as an important sink for ENMs and as a huge microbial reservoir (Zhou et al., 2021). Considering the important role of soil microorganisms in plant growth and soil ecosystem stability, the interactions between 2D-MoS_2 and soil microbial communities need to

be evaluated. Bae et al. (2021) co-cultured two isolated soil beneficial bacteria *Bacillus cereus* and *Pseudomononas aeruginosa*, with 2D-MoS_2 and its precursor form in an artificial liquid medium. Using an assessment criterion of EC_{50} values, it was found that the pristine exfoliated 2D-MoS_2 were significantly more toxic to both bacteria than their bulk form. In a real soil environment, metagenome-based results showed that 2D-MoS_2 (10 or 100 mg/kg) exert no negative effect on the soil microbial community of rice (Zhao et al., 2022). Besides, novel 2D-MoS_2-related fungicides have been gradually developed to promote crop growth and improve resistance, as summarized in Table S2.

In addition to ecological aspects, the antimicrobial properties of 2D-MoS_2 offer unprecedented opportunities for biomedical applications. The conventional antibacterial mechanism of 2D-MoS_2 could be summarized into two pathways, ROS-mediated oxidative stress and loss of membrane integrity through mechanical damage or extraction of phospholipid molecules (Kumar et al., 2022; Shang et al., 2017; Wu et al., 2018). As an extension of the antibacterial mechanism, Shi et al. (2021) recently reported that nanopores on 2D-MoS_2 could effectively disrupt membrane structures by enhancing electron transport with biological membranes. This excellent antimicrobial performance was further validated in cases of intestinal and ocular infections in mice. Along this line, attempts to enhance antimicrobial activity by modifying or functionalizing 2D-MoS_2 have gradually come to be developed. These functionalized materials include MoS_2/TiO_2 multidimensional heterostructures (Dong et al., 2022; Yan et al., 2020), silver-doped MoS_2 structures (Xu et al., 2021; Yang et al., 2018), MoS_2-Cu nanoparticle composite structures (Li et al., 2020), MoS_2-EDTA systems, etc (Fan et al., 2015a). The enhanced antibacterial mechanism could be concluded as increased ROS production and oxidase-like activity as well as more significant membrane disruption. Information on antimicrobial studies related to 2D-MoS_2, including heterogeneous structures, evaluation endpoints, and antimicrobial mechanisms, is detailed in Table S2.

3. Nature transformation of 2D-MoS_2 in environments

3.1. Natural aging mediated by abiotic factors

3.1.1. Photo-aging

2D-MoS_2 released into the environment undergo a series of aging processes driven by environmental or biological factors, where transformation occurs (Figure 2). Photo-aging is an important natural aging pathway and therefore the most common approach for simulating the 2D-MoS_2 aging process in the laboratory (Shi et al., 2021). UV light can accelerate the oxidation of crystal substrates of 2D-MoS_2, resulting in phase changes and surface defects (Cao et al., 2021b). Solar radiation also contains a visible light component. Visible light irradiation of 2D-MoS_2 at 400–800 nm (500 W) for one hour was reported to induce nanopore generation (Tong et al., 2019). In terms of environmental behavior, visible light aging could reduce the colloidal stability of 2D-MoS_2. Although the aging simulations are feasible from a laboratory point of view, doubts remain as real environmental aging processes occur not alone, but in conjunction with several environmental factors. For example, in an aqueous environment with high oxygen content, visible light could competitively capture electrons on 2D-MoS_2 in concert with dissolved oxygen and promote their chemical dissolution through free radical chain reactions (Zou et al., 2019). In addition, visible light synergistically promoted the aggregation of 2D-MoS_2 in the presence of cations (Liu et al., 2021). This suggests that the environmental stability of 2D-MoS_2 is co-regulated by multiple factors, again emphasizing the necessity for synergistic aging tests. Shi et al. (2021) simulated synergistic aging of 2D-MoS_2 under laboratory conditions using UV (365 nm,150 W, 24 h) and Fenton reactions (1 mM $FeSO_4$:50 mM H_2O_2=1:10, 4 h). This UV-Fenton reaction system generated hydroxyl radicals that attack 2D-MoS_2 to induce nanopores. This was an important attempt to assess 2D-MoS_2 co-aging, but more complex co-aging scenarios need to be further explored.

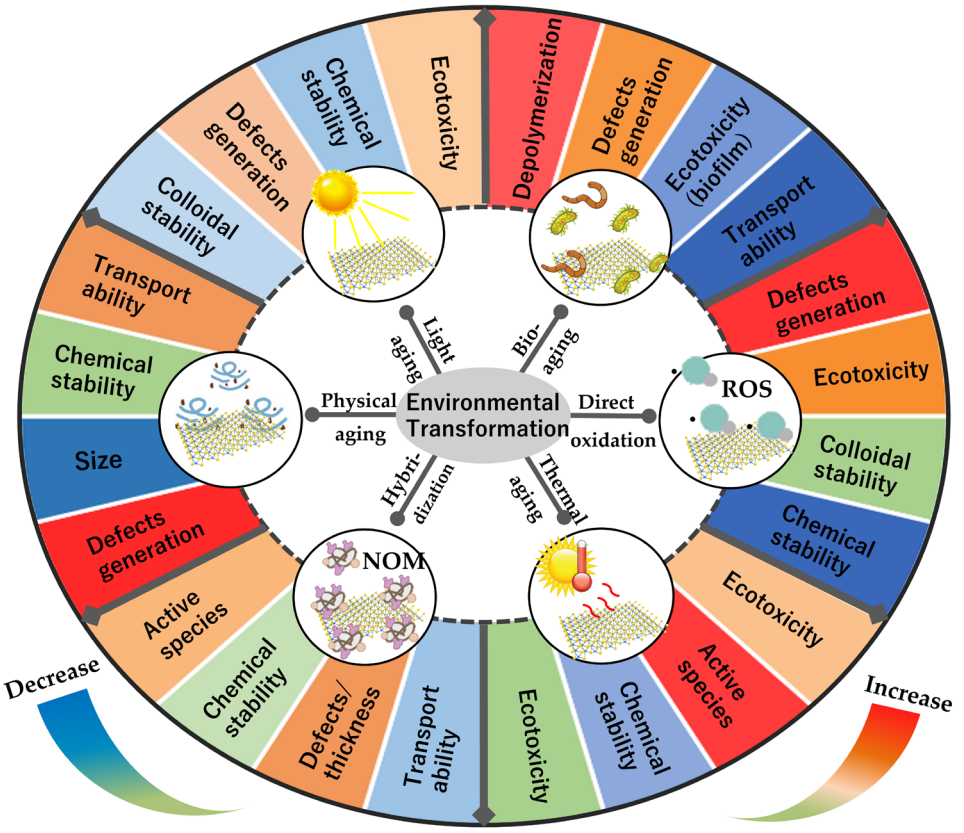

Figure 2. Natural aging pathways and concomitant changes in the physicochemical properties and the toxicity profile of 2D-MoS_2.

3.1.2. Humic acid

Natural organic matter (NOM) is widely present in surface water (Han et al., 2019; Louie et al., 2015). NOM can interact with ENMs through direct (hybridization) or indirect (radical-mediated) pathways, thus changing the properties and environmental fate of the ENMs (Chowdhury et al., 2014). The carbon functional groups of humic acid (HA) were grafted onto monolayer 2D-MoS_2 *via* exposed sulfur atoms after hybridization at 10, 50, and 100 mg/L HA with 2D-MoS_2 for 56 days (14:10 light-dark ratio) (Zou et al., 2018). The hybridization process resulted in smoother edges, simultaneous changes in layer structure (monolayer to multilayer) and crystalline phase (2H to 1 T), and a significant increase in thickness (~2–10 nm) of 2D-MoS_2. In addition, HA hybridization accelerated Mo release under light conditions but led to increased colloidal stability of 2D-MoS_2. Lee et al. (2019) reported similar results and further revealed a high correlation of this process with light radiation, as HA extracted from Suwannee River retarded the oxidative degradation of 2D-MoS_2 in the dark.

3.1.3. Extracellular polymeric substances

As an important component of native NOM and a major reservoir of organic carbon, extracellular polymeric substances (EPS) secreted by microorganisms and algae are prevalent in the environment (Xiao & Zheng, 2016). EPS readily interact with ENMs released into the environment, forming an environmental corona and further mediating changes in their physicochemical properties and environmental fate (Naveed et al., 2019). The hybridization with EPS can lead to distortion of the layered edges of 2D-MoS_2 and the appearance of SVs and nanopores on the

basal plane (Zeng et al., 2022). The chemical stability of 2D-MoS_2 increased, and the catalytic activity decreased after hybridization with EPS, but there was no significant phase transition (1 T phase dominated). Meanwhile, EPS hybridization alleviated the aggregation potential of 2D-MoS_2. Recently, EPS has been shown to alter the ability to generate ROS in 2D-MoS_2 suspensions. At this point, the existing research findings are divergent. Zou et al. (2021b) found that EPS hybridization at 10 mg C/L led to a decrease in the cutoff wavelength (1242.375/bandgap) and the light utilization efficiency of 2D-MoS_2, thus reducing the ability to generate ROS species in the MoS_2-EPS system. Another study yielded the opposite result, and showed that the interaction of EPS with 2D-MoS_2 enhanced the ability of the system to generate free radicals, including ·OH (up-regulated by 22%), $\cdot O_2^-$ (up-regulated by 91%), and $^1O_2$ (up-regulated by 18%) (Zeng et al., 2022). The lack of information on the concentration of EPS makes it difficult to compare these contradictory results. Further development of a more standardized approach is needed to clarify the contribution of EPS to ROS generation in 2D-MoS_2.

3.1.4. Nanocolloids

Nanocolloids are widely present in environmental media, especially in water, and will inevitably interact with 2D-MoS_2 released into the environment. Zeng et al. (2021) simulated the binding of nanocolloids (8.7 ± 0.7 nm) extracted from river and sea water with 2D-MoS_2. The nanocolloids could adsorb significantly to the surface of the 2D-MoS_2, which led to an increase in 2D-MoS_2 thickness (~1.3–3 nm) and sharper edge morphology. Compared to HA, the adsorption of nanocolloids on monolayer MoS_2 was more pronounced, and the formed nano-colloid surface coating promoted the dissolution of 2D-MoS_2 (6.1–11.3%) in water. Unlike aqueous solutions, the nanocolloids inhibited the Mo species release from 2D-MoS_2 in BG-11 system, an algal and protozoan culture medium rich in Na^+ and K^+ elements. The specific mechanisms by which nanocolloids promote or inhibit the release of ions from 2D-MoS_2 in different media are still poorly understood. To date, comprehensive laboratory and field *in situ* studies of NOM interactions with 2D-MoS_2 remain limited and insufficient to generalize universal rules.

3.1.5. Mechanical force and other nonselective aging

As with most contaminants, 2D-MoS_2 released into environment media may first mechanically interact with soil particles or water flow. Such direct natural aging may lead to surface disruption and fragmentation of 2D-MoS_2, which contributes to further interaction of various substances with 2D-MoS_2, thus further accelerating the aging process. The widespread presence of sulfide and ozone in the environment may contribute to the direct oxidation of 2D-MoS_2. In extreme environments such as deep soil and subsea, high temperature, high salt, strong acid, and alkali may also lead to rapid aging of 2D-MoS_2.

3.2. Transformation mediated by biological factors

In addition to natural aging factors, 2D-MoS_2 released into the environment may be ingested by various organisms. Biological behaviors such as chewing can cause the fragmentation of 2D-MoS_2 due to physical stress. Specific digestion and catabolic processes in organisms may also lead to the mineralization or assimilation of 2D-MoS_2. It has been reported that 2D-MoS_2 enriched in rice roots and mouse liver can be transformed from Mo (IV) to Mo (VI) by bio-enzymatic processes, and the transformation rate is usually higher than 80% (Cao et al., 2021a; Zou et al., 2023). Degraded 2D-MoS_2 is usually involved in coenzyme synthesis and metabolic processes in ionic forms. More comprehensive work is still needed in the future to trace the transformation patterns and specific mechanisms of 2D-MoS_2 in highly complex organisms.

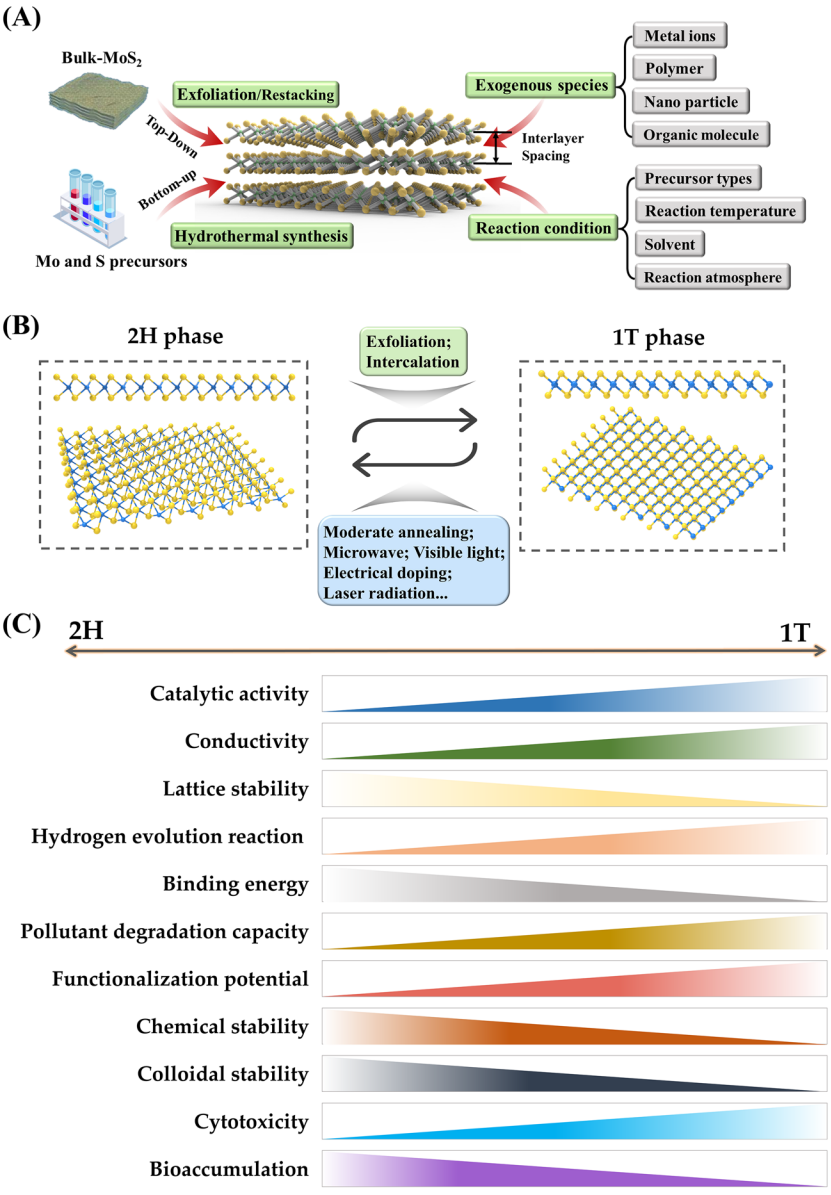

Figure 3. The synthetic approaches of 2D-MoS_2 (A). two typical lattice structures and phase transition pathways of 2D-MoS_2 (B). changes in physicochemical properties and toxicity accompanying the 2D-MoS_2 phase transition (C).

4. Engineering design of 2D-MoS_2

In addition to the natural aging experienced after releasing into the environment, 2D-MoS_2 also possess excellent artificial modification potential due to their design flexibility. In this section, we summarize the engineering design pathways of 2D-MoS_2 and corresponding changes in physicochemical properties (Table S3).

4.1. Interlayer design

The performance of 2D-MoS_2 is largely dependent on its layered structure. Therefore, the interlayer design of 2D-MoS_2 has been emerging in recent years to expand its applications in water

purification, electrocatalysis, and energy storage (Chen et al., 2017; Gong et al., 2019; Sun et al., 2020, 2017). The interlayer editing can be achieved through "top-down" or "bottom-up" approaches during *in situ* synthesis or subsequent tailoring (Rasamani et al., 2017), as schematically summarized in Figure 3(a).

4.1.1. Top-down exfoliation

As concluded in previous classical reviews, the weak van der Waals forces between layered structures of bulk-MoS_2 allow them to be ideal interlayers for various intercalators (Ai et al., 2016; Benavente et al., 2002). The monolayers of MoS_2 may re-stack into fewer layer structures during exfoliation. In this process, alien species are confined in the interlayer gaps, resulting in interlayer-expanded 2D-MoS_2 (Rasamani et al., 2017). Existing reported intercalators include metal ions, organic molecules and polymers, and nanoparticles (Figure 3(a)) (Goloveshkin et al., 2015; Huang et al., 2013). The selection of different foreign intercalators has a significant effect on the layer spacing regulation of 2D-MoS_2. The interlayer spacing of 2D-MoS_2 obtained by intercalation of Li, Na, and K could be increased from the original 0.615 nm to 0.708, 0.899, and 0.935 nm, respectively (Jeong et al., 2015; Zheng et al., 2014). This was attributed to crystal structure changes due to electron transfer between the intercalator and 2D-MoS_2 (Voiry et al., 2013). An increase in the molar ratio of the intercalator polyethylene oxide to MoS_2 from 0.25 to 1 resulted in a corresponding increase in the interlayer spacing of 2D-MoS_2 from 1.19 to 1.45 nm, respectively (Liang et al., 2015). This suggests that precise regulation of interlayer spacing could also be achieved by adjusting the molar ratio of intercalator to MoS_2.

4.1.2. Bottom-up synthesis

The synthesis of 2D-MoS_2 using hydrothermal methods with different precursors of Mo and S is widely applied due to its ease of operation and mass production (Xu et al., 2020b). The layer spacing of 2D-MoS_2 can be regulated to some extent by controlling the reaction conditions, including precursor type, reaction temperature, solvent, and atmosphere (Figure 3(a)). The currently reported 2D-MoS_2 synthesized *via* this "bottom-up" approach possess a layer spacing ranging from 0.65 to 1.45 nm (Gao et al., 2015; Rasamani et al., 2017; Xie et al., 2013).

4.2. Phase regulation

According to the difference in atom-stacking configurations of Mo and S, the bulk form of MoS_2 can exhibit stacking polymorphism (Chhowalla et al., 2013; Wang & Mi, 2017). Specifically, the semiconducting 2H phase is coordinated in the form of a triangular prism, while the metal 1 T phase is coordinated in an octahedral structure (Figure 3(b)) (Enyashin et al., 2011). Under natural conditions, the 1 T phase can easily transform into the 2H phase and then exist stably (Wang et al., 2012). The stable 2H phase can only transform to the metastable 1 T phase upon specific artificially induced conditions (Eda et al., 2011; Wypych & Schöllhorn, 1992). Chemical intercalation can achieve the above-mentioned phase transition regulation while performing interlayer extension of 2D-MoS_2. UV-Vis absorption spectroscopy and XPS spectroscopy indicated that the 2H characteristic peaks of 2D-MoS_2 chemically exfoliated using alkali metal ions were weak, while the 1 T characteristic peaks were dominant (60–70%), and the binding energy was about 0.9 eV lower than that of the 2H phase (Wang et al., 2016a). These characteristics represented the 2H to 1 T phase transition that occurred by exfoliation, which has recently been widely applied as an effective strategy to improve the catalytic activity of 2D-MoS_2 (Voiry et al., 2016, 2013). 2D-MoS_2 synthesized by the "bottom-up" method is typically a mixture of 1 T/2H with a variable phase ratio. The pure 2H-phase 2D-MoS_2 are normally obtained by mild annealing. For example, Eda et al. (2011) reported that the thermodynamically stable 2H phase could be fully recovered by natural annealing at 300 °C for

60 min (5 °C/min). The 1 T to 2H phase transition can also be induced by laser radiation, microwave, and visible radiation, but this may be accompanied by other changes, such as decreased catalytic activity and conductivity, etc (Figure 3(c)) (Eda et al., 2011; Fan et al., 2015b; Xu et al., 2016).

4.3. Defect engineering

4.3.1. Nanopores

Surface design and reaction are important ways to improve the nanomaterial properties (Zhang et al., 2022a, 2022b). The creation of nanopores on the 2D-MoS_2 basal plane can easily be achieved by parameter control during the *in situ* synthesis or by subsequent aging using UV or visible light irradiation (Shi et al., 2021; Tong et al., 2019). As an important aspect of defect engineering, the creation of nanopores has greatly expanded the application of 2D-MoS_2 in several fields. Firstly, these nanopores can act as electron donors, thus enhancing the electron transport between 2D-MoS_2 and biofilms (Shi et al., 2021). This holds promise for the development of a new generation of nano-based antibacterial products. Secondly, the porous structure can provide a higher edge-to-atom ratio compared to the intact 2D-MoS_2, thus greatly improving their catalytic efficiency (Su et al., 2018). Recently, great efforts have been made to finely regulate the size of nanopores, as the smaller the diameter and sub-nanometer thickness of the nanopores, the more advantageous it is for applications in DNA analysis, protein detection, sequencing, and biosensing (Wu et al., 2022). Smaller size or individual nanopores are usually created *via* electric pulse, probe microscope etching, and ion beam radiation (Kuan et al., 2015; Schneider et al., 2013). The specific mechanism is the induction of pore generation by a local electrochemical reaction generated at the tip (Huang et al., 1992). Feng et al. (2015) prepared nanopores (<1 nm) by introducing electrochemical reactions at single-atom vacancies, followed by successive removal of individual atoms from the layered MoS_2 lattice. However, it is currently difficult to modulate the size, density, and location of nanopores at the same time.

The generation of nanopores is usually accompanied by interrelated changes in the physicochemical properties of 2D-MoS_2. The creation of nanopores increases 2D-MoS_2 thickness (e.g. from 0.95 ± 0.15 nm to 1.26 ± 0.23 nm) and triggers a crystalline phase transition from the metastable 1 T phase to the thermodynamically stable 2H phase (Tong et al., 2019). The photoluminescence intensity of porous 2D-MoS_2 was enhanced by about 35% compared to its pristine form due to oxygen adsorption-induced heavy *p* doping and trion-exciton conversion (Nan et al., 2014; Tong et al., 2019). Moreover, nanopores can cause an uneven distribution of electron density and an increase in the crystal plane spacing (0.24–0.30 nm) of 2D-MoS_2 (Tong et al., 2019).

4.3.2. Surface cracks

Similar to nanopores, surface cracks are reported to cause enhanced photoluminescence of 2D-MoS_2, thus greatly expanding its prospects for optoelectronic applications (Matioli et al., 2012). Nan et al. (2014) induced cracks on the sheet structure of 2D-MoS_2 by a high-temperature annealing process. The cracks exhibited abundant dangling bonds of Mo and S atoms. The aggregation of these atomic vacancies can act as active molecular adsorption centers and significantly increase the photoluminescence intensity at the defect site.

4.3.3. Sulfur vacancies

Unlike aforementioned nanopores and surface cracks, atomic vacancies are a more stable defect structure (Zhou et al., 2013). The sulfur atoms on the 2D-MoS_2 substrate can be removed by annealing (400–800 °C) in a hydrogen atmosphere (with 5% argon) (Li et al., 2019; Zou et al., 2021a). Notably, the point defects tend to form below 600 °C and produce Mo-S dangling bonds; at temperatures above 600 °C, the S atoms in the lattice were heavily stripped to form ultrarich

defects (Li et al., 2019). Local SVs can also be induced by plasma irradiation and high-temperature oxygen doping (Komsa et al., 2012). However, these methods are not suitable for mass production due to the directional nature of an argon plasma. To address this issue, Tsai et al. (2017) recently used an electrochemical method to hydrogenate the sulfur atoms on the MoS_2 substrate and detach them from the system in the form of H_2S. This approach is highly scalable as the amount of SVs can be adjusted by the magnitude of the desulfurization voltage and controlling the applied time.

Compared to the intact form, SV-enriched 2D-MoS_2 are more capable of generating free radicals, which is attributed to the enhanced photo-activity due to the change in the band structure (Splendiani et al., 2010). SVs could induce photoluminescence enhancement and increase electrical conductivity *via* a different mechanism from nanopores and surface cracks. Specifically, SVs could provide additional empty states near the Fermi level and induce spin orbital splitting of the valence band and direct excitonic transitions, thus enhancing carrier density (Qiu et al., 2013; Splendiani et al., 2010; Wang et al., 2021). Moreover, the creation of SVs could interrupt the stable covalent bonds of 2D-MoS_2 and provide additional active sites, which could largely alter their stability and mechanism of biological interaction (George et al., 2012; Tong et al., 2019). Therefore, it is necessary to evaluate the effects of SVs on the ecotoxicity of 2D-MoS_2, which is crucial for the sustainable development and application of defect engineering in 2D-MoS_2.

5. Impacts of nature transformation and engineering design of 2D-MoS_2 on its toxicity and mechanisms of action

5.1. Size and layer structure

Exfoliation from the bulk form is the most used method to obtain 2D-MoS_2. The flexible regulation of the exfoliation process allows the 2D-MoS_2 obtained to be diversified in terms of size and thickness. Chng et al. (2014) exfoliated 2D-MoS_2 with different thicknesses using three different intercalators (*tert*-butyl lithium, n-butyl lithium, and methyl lithium). It was demonstrated in a test on A549 cell lines that the ability to induce loss of cellular activity was positively correlated with the number of 2D-MoS_2 layers, which may attribute to the increase in the active edge sites and surface area of the nanosheets (Chng et al., 2014). The layer effects are supported by another study reporting cellular internalization of 2D-MoS_2. The thicker 2D-MoS_2 (40 layers) were internalized by the cells while the thinner 2D-MoS_2 (5 layers) were mostly blocked at the cell surface upon the same exposure concentration defined by the specific surface area (Zhou et al., 2019b). In the same report, it was found that both cell surface adhesion of thinner 2D-MoS_2 or significant internalization of thicker 2D-MoS_2 induced cellular autophagy in the same mTOR-dependent pathway (Zhou et al., 2019b). This suggests that there may be a common regulatory mechanism behind the significantly different phenotypic changes. We anticipate that the use of emerging omics tools will hold promise for further clarification of these common and specific regulatory mechanisms.

The size of nanomaterials is usually an important parameter that affects their biological effects (Zhou et al., 2019a). It was reported that 2D-MoS_2 of different lateral sizes (~50 nm, ~117 nm, and ~177 nm) internalized in three human cell lines (A549, AGS, and THP-1) without significant subcellular damage or other size-dependent cell death (Moore et al., 2017). Subsequent cellular inflammation assays showed an effect of 2D-MoS_2 size, with the smallest 2D-MoS_2 causing the most significant rise in THP-1 cytokines (Moore et al., 2017). However, this result was obtained considering endotoxin contamination, which means that it cannot be determined whether endotoxin mediated the more intense cellular inflammatory response induced by small-sized 2D-MoS_2. Although several studies used 2D-MoS_2 with different sizes, the lack of a consistent experimental design prevented us from directly comparing the contribution of size effects on toxicity. In comparison, a recent study on the microalgae *Chlorella vulgaris* compared the differences in toxicity mechanism of 2D-MoS_2 at the nanoscale (~400 nm) and micron scale (~3.6 μm) (Zou et al., 2020). Nanoscale 2D-MoS_2 were more toxic to algal growth, antioxidant enzyme

activity, MMP, and lipid peroxidation. 2D-MoS_2 with a smaller lateral size caused more severe surface shrinkage and depression of the microalgae, although these changes were not persistent. In contrast, micron-sized 2D-MoS_2 led to more severe and persistent cell membrane and chloroplast damage. The combination of proteomics and metabolomics further revealed that size-specific toxicity was associated with intracellular transport, acetyl coenzyme A biosynthesis, carbon fixation, and carbohydrate metabolism. These results clearly indicate that the design and application of 2D-MoS_2 should fully consider the role of size effects in its ecotoxicity.

Overall, the role of size and thickness on the toxicity of 2D-MoS_2 has begun to attract attention, but still lack mechanistic explanations. Furthermore, although smaller-size ENMs (e.g., quantum dots and silver nanoparticles) are more rapidly excreted from the body, it remains unknown whether this rule is also followed under 2D-MoS_2 exposure. Based on the existing reports, the *in vivo* distribution and fate of 2D-MoS_2 with different sizes and thicknesses should be further clarified in the future.

5.2. Phase transition

The evaluation of 2D-MoS_2 toxicity requires consideration of its flexible phase transition. Researchers investigated the biological effects of two different phases of 2D-MoS_2 on *Nostoc sphaeroides*. Although preferential dissolution of the 1 T phase was observed, the promotion of C/N metabolism and biochemical synthesis of *Nostoc sphaeroides* by 2D-MoS_2 was attributed to its catalytic and photoelectric activity, independent of the released Mo species (Chen et al., 2021; Zou et al., 2019). In this case, the 2H-phase 2D-MoS_2 induced a more pronounced upregulation of carbon-related and amino acid-related metabolites than 1 T-phase nanosheets at all concentrations tested, suggesting a greater ability of 2H-phase 2D-MoS_2 to reprogram cyanobacterial metabolism. In the antibacterial field, 2D-MoS_2 with 1 T phase have higher sterilization activity than 2H phase due to its higher electronic conductivity (Fan et al., 2015a). Therefore, regulating the ratio of 1 T phase is a potential strategy to enhance the antibacterial efficacy of 2D-MoS_2. Special attention needs to be paid to the direct release of 2D-MoS_2 with a high 1 T phase ratio into the environment and their phase conversion driven by environmental factors, as this may exacerbate their reactivity with sensitive biota. For instance, the 1 T phase of 2D-MoS_2 was more toxic to microalgae (*Chlorella vulgaris*) than the 2H phase, as evidenced by enhanced growth inhibition, oxidative damage, cytoplasmic lysis, and photosynthetic interference (Zou et al., 2020, 2019). This change in toxicity was independent of the release of ions but was attributed to the regulation of amino acid and unsaturated fatty acid metabolism. In addition, the chloroplast ultrastructural damage and the increase in membrane permeability induced by the 1 T phase of 2D-MoS_2 were more persistent on *Chlorella vulgaris*. These toxicity results were obtained from exposure comparisons of pure 1 T or 2H materials, lacking systematic control of phase ratios. Further experiments are required to understand the contribution of phase ratio changes in 2D-MoS_2 to their accumulation, excretion, and biotransformation in mammals and other organisms before translation into biomedical and antimicrobial products.

5.3. Surface defects

At this stage, the toxicological evaluation of 2D-MoS_2 has begun to focus on the contribution of surface defects and has provided valuable information. It has been reported that annealing-induced SVs *via* a hydrogen-argon atmosphere significantly enhanced the toxicity of 2D-MoS_2 to *Chlorella vulgaris*, as evidenced by greater oxidative stress (29.6–123.7% for ROS and 25.6–175.2% for malondialdehyde) and more severe chloroplast ultrastructural damage (Zou et al., 2021a). The significant toxicity-enhancing effect may be related to SV-mediated radical production and decreased colloidal stability of 2D-MoS_2. Interestingly, when the atomic vacancies were further expanded or aggregated to form nanopores, their toxicity-promoting effects on the

same aquatic microalgae were reversed (Tong et al., 2019). This nanopore-mediated mitigation of aquatic toxicity was characterized as the alleviation of ultrastructural damage (cell shrinkage and wall rupture) and metabolic disturbances in microalgae cells. The specific mechanism by which surface defects regulate 2D-MoS_2 toxicity needs to be further determined. Shi et al. (2021) recently reported the high destruction efficiency of nanopore-enriched 2D-MoS_2 on biofilms. The corresponding toxicity mechanism was accurately described as nanopore-enhanced electron transport between the nanomaterial and biofilms. Atomic vacancies also played a key role in another recently reported mechanism of 2D-MoS_2-induced cytosolic iron sagging toxicity. The lysosomal damage caused by 2D-MoS_2 in the cytoplasm of human BEAS-2B and THP-1 cells was reduced after shielding off the atomic vacancies by surface passivation (Xu et al., 2020a). Accordingly, Fe^{2+} release and ferroptotic cell death were also reduced. These two studies represent an important expansion of the defect-mediated toxicity mechanism of 2D-MoS_2.

Despite the current enthusiasm to modify the properties of 2D-MoS_2 by defect engineering, studies to evaluate the role of these defects in MoS_2 toxicity are still in their infancy. We found limited information on the toxicity assessment of 2D-MoS_2 involving the regulation of surface defects using mammals, aquatic vertebrates, and soil organisms. Moreover, dispersed atomic vacancies on the basal plane and large size aggregated nanopores contribute differently and sometimes contrarily to 2D-MoS_2 toxicity. This suggests that future toxicity evaluation models need to incorporate precise regulation of defect characteristics to determine whether these defects have shape- or size-specific effects on nanosheet toxicity.

5.4. Hybridization of natural organic matter

The ubiquitous presence of NOMs in the aqueous environment has been shown to significantly modify the aquatic toxicity of 2D-MoS_2. Hybridization with HA (10–100 mg/L) significantly alleviated the inhibition of cell division and damage to chloroplast ultrastructure by 2D-MoS_2 in *Chlorella vulgaris* (Zou et al., 2018). In this case, the internalization of 2D-MoS_2 by microalgae cells was reduced due to the increased thickness of 2D-MoS_2 caused by hybridization, which further alleviated the cytoplasmic membrane contraction and cytoplasmic dissolution. Unlike HA, the presence of nanocolloids (10 mg/L) significantly exacerbated the inhibition of *Chlorella vulgaris* growth by 2D-MoS_2 and their internalization (Zeng et al., 2021). The enhanced cell growth inhibition may be attributed to the abundance of metals and polycyclic aromatic hydrocarbons in the nanocolloids, while the use of different pharmacological inhibitors showed that the promoted internalization was by nanocolloid-mediated macrophage endocytosis (Zeng et al., 2021). Furthermore, the mixed system of nanocolloids and 2D-MoS_2 specifically caused distortion and deformation of *Chlorella vulgaris* cells. The adsorption of EPS (~120 mg/g) on 2D-MoS_2 caused more significant inhibition of growth and cell morphological damage to *Chlorella vulgaris* than 2D-MoS_2 exposure alone (Zeng et al., 2022).

The hybridization with different NOMs resulted in fully different aquatic toxicity changes of 2D-MoS_2, suggesting that different deep-seated toxicity mechanisms may exist. This is confirmed by the application of metabolomics and transcriptomics. Hybridization of nanocolloids and 2D-MoS_2 induced more severe metabolic disorders in *Chlorella vulgaris*, including biosynthesis of isoquinoline related to membrane permeability and amino acid metabolic processes (Zeng et al., 2021). In comparison, the mechanism of EPS-induced toxicity enhancement is associated with the process of amino acid and fatty acid biosynthesis (Zeng et al., 2022). As organic substances widely distributed in the aqueous environment, there are many types of NOMs, including grease, saccharides, proteins, natural rubber, etc. In assessing their effects on the toxicity of 2D-MoS_2, further refinement of NOM types is needed to quantify the proportional contribution of the various components. Scenarios simulating natural aquatic environments also need to be considered based on existing studies, including but not limited to the use of real surface water conditions, setting broader pH ranges, etc.

5.5. Surface functionalization and heterogeneous structure coupling

Surface functionalization and heterostructure coupling are considered valuable attempts to ensure the superior performance of 2D-MoS_2, but the associated risk profile changes need to be understood accordingly. PEG-functionalized 2D-MoS_2 are reported to possess higher enzyme-like catalytic activity, greater water dispersibility, and lower cytotoxicity than its original form (Kou et al., 2014; Liu et al., 2015; Zhao et al., 2017). PEG-MoS_2 also have a faster excretion rate *in vivo* (Hao et al., 2017). Similarly, both thiobarbituric acid (TBA) and poly(lactic-co-glycolic acid) (PLGA) coating can improve the solubility and biocompatibility of 2D-MoS_2 (Rosli et al., 2018; Wang et al., 2015b). These findings facilitate the accelerated application of 2D-MoS_2 in biomedical fields. In addition to surface coating, creating heterogeneous structures by introducing exogenous intermediates is now a common modification approach. Zhou et al. (2022) reported that 2D-MoS_2 doped with graphene oxide quantum dots (GOQDs) possess enhanced colloidal and chemical stability and can alleviate LPS-induced apoptosis and membrane damage in human embryonic lung fibroblasts (HELFs). Activation of the Wnt/β-catenin pathway and related immune factors is one of the cytoprotective mechanisms of MoS_2-GOQDs heterogeneous structure (Zhou et al., 2022). In terms of environmental risk, the toxicity of 2D-MoS_2 to *Chlorella vulgaris* was significantly reduced once coupled with environmentally abundant thiols such as L-cysteine and thioglycolic acid. Mechanistically, thiols can alleviate MoS_2-induced inhibition of algal division, oxidative stress, disruption of photosynthesis, and metabolic disorders, and synergistically alter the interaction between 2D-MoS_2 and adsorbed intracellular proteins to reduce their phytotoxicity (Zou et al., 2022). However, it was also reported that chitosan-functionalized 2D-MoS_2 have a series of adverse effects on human dermal fibroblasts (HDFs), including disturbance of membrane homeostasis, DNA damage, oxidative stress, apoptosis, and impaired energy metabolism (Yu et al., 2017). Toxicological data obtained using zebrafish (*Danio rerio*) similarly highlight the potential effects of chitosan-functionalized 2D-MoS_2, including lesions, inflammation, apoptosis, and oxidative damage in the gills and liver sites (Yu et al., 2018). Boronic acid functionalization significantly enhances the destructive interactions between 2D-MoS_2 and bacterial (*Escherichia coli* and *Bacillus cereus*) and nematodes (collected from a slaughterhouse) (Sattari et al., 2020). The possible mechanism is that the boronic acid functional group can bind to glycoproteins on the bacteria and nematode surfaces, amplifying the multivalent covalent interactions between 2D-MoS_2 and biofilms. Meanwhile, the boronic acid functional group also covers the nematode pores and blocks the transport and uptake of nutrients, ultimately interfering with metabolism. Similarly, 2D-MoS_2 co-modified with chitosan and cyclic peptide have been reported to significantly increase the photothermal-mediated apoptosis of HCT-116 cells through the inhibition of heat shock proteins (Hsp90) (Ariyasu et al., 2017). It is thus clear that not all modifications of 2D-MoS_2 are favorable for their biosafety, especially in the biomedical and ecological fields. Future attempts are required to develop 2D-MoS_2-based composite nanomaterials with both excellent engineering performance and environmental separation properties, for example, by loading them onto macro carriers or encapsulating them into eco-friendly biopolymers. Meanwhile, fully assessing the potential risks of existing nano-composites is also an urgent task.

6. Environmental implication and future outlook

After systematically generalizing the available data, we discuss here the shortcomings of the currently available studies and priorities for future research.

i. As 2D-MoS_2 show great potential for biomedical applications, more stringent biosafety standards should follow. Most of the current biocompatibility assessments are based only on simple quantification of cell viability and lack deep mechanistic exploration. The existing reports on the cytotoxicity of 2D-MoS_2 are contradictory, suggesting that no universal conclusions should be drawn from any single experimental result. Most importantly, all *in*

vitro toxicity data need to be fully validated *in vivo*. We recommend more in-depth mechanistic studies, including but not limited to the mechanisms of cellular internalization and intracellular fate of 2D-MoS_2, molecular profiling at the metabolic and transcriptional levels, and thorough validation *in vivo*. Meanwhile, the establishment of uniform exposure procedures to obtain comparable toxicity data is crucial for the safe application of 2D-MoS_2 in the biomedical field.

ii. Given the potential release due to the widespread application of 2D-MoS_2, environmental exposure pathways require urgent investigation. To achieve this goal, a global survey of environmental concentrations of 2D-MoS_2 is needed to understand environmentally relevant exposure concentrations. Toxicity assessment models based on aquatic unicellular algae such as *Chlorella vulgaris* and *Nostoc sphaeroides* have been developed, and biological responses at the metabolic, protein, and transcriptional levels have been revealed with emerging omics tools. Beyond that, there are limited toxicity data for other more complex organisms. Therefore, more comprehensive toxicity assessment models need to be applied to cover all environmental receptors, including fish, benthic organisms, soil invertebrates, mammals, etc. Long-term exposure experiments are recommended, including whole-life cycle monitoring, food chain transport, and multi-generational toxicity studies.

iii. When different specific parameters (e.g., size, layer structure, surface morphology, etc.) were considered in the toxicity assessment, the results often significantly differed or were even contradictory (Figure 4). We recommend the incorporation of cross-disciplinary approaches that interrelate specific biological effect endpoints with material characteristics and validate the correlation. Based on the available data, further attention needs to be paid to the effects of natural aging factors on the environmental behavior, fate, and toxicity of 2D-MoS_2. Most of the current studies have focused on aquatic media and lack information

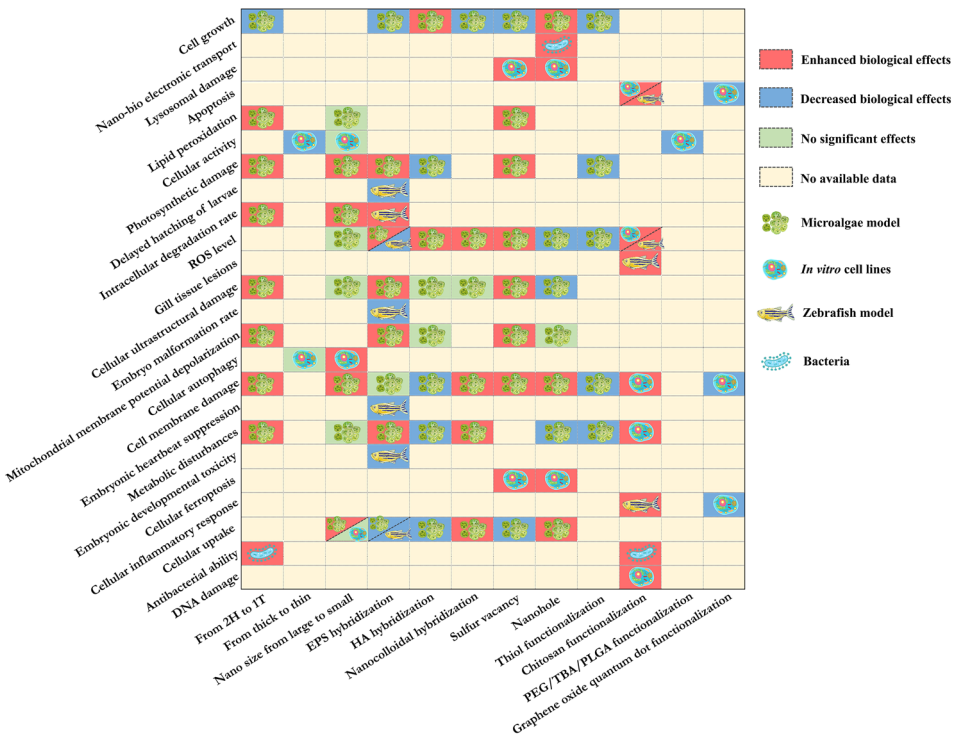

Figure 4. Correlation of 2D-MoS_2 transformation pathways and toxicity assessment results as determined by different biological models.

on the potential transformation pathways and migration behavior of 2D-MoS_2 in other environmental compartments. Moreover, testing the aging of 2D-MoS_2 should not be limited to laboratory conditions, as the natural environment tends to lead to a composite multi-factor aging process with discontinuous intensity. We suggest studying 2D-MoS_2 aging in more realistic environmental scenarios, such as in natural surface waters or field soils, monitoring changes in the physicochemical properties of 2D-MoS_2 at realistic time scales.

iv. The surface functionalization or coupling of heterostructures helps to enhance the intrinsic properties of 2D-MoS_2, but this process inevitably introduces potentially exogenous contaminants. 2D-MoS_2 released into the environment may also co-exist with other environmental contaminants (metals, organic pollutants, and other ENMs, etc.). Considering the potential co-exposure scenarios, more work is urgently needed to accurately understand the combined biological effects of 2D-MoS_2 with other contaminants. Additionally, the secondary contamination caused by intercalator leakage (especially lithium compounds) also deserves attention.

v. With the rapid expansion of 2D-MoS_2 applications, redesign, and functionalization methods will continuously emerge. Therefore, the development of effective toxicity prediction models is an urgent task. The integration of machine learning into these models for a more intelligent parameter organization and fitting process is promising. The ultimate goal is to rapidly predict the toxicity and develop mitigation options to accelerate the commercial application of 2D-MoS_2.

In conclusion, the widespread use of 2D-MoS_2 must be fully weighed against its potential health risks. It is premature to promote large-scale commercial applications until the toxicity characteristics of 2D-MoS_2 are fully described and the crucial role of engineered or environmental induced transformations in that toxicity profile are clearly identified.

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