A rapidly developing approach aiming at minimizing ENM associated health and environment risk is via the use of biological entities (as such or their extracts) to design desired nanomaterials. The biologically synthesized nanomaterials are hypothesized to be more benign towards human health and environment as compared to chemically synthesized nanomaterials [20], but a detailed safety analysis and risk assessment is a big research lacuna at present. ENMs synthesized through biological routes (biogenic ENMs) have an additional matrix of biomolecules on the surface by virtue of the synthesis method (Figure 1). The biomolecular components such as proteins, carbohydrates, phytochemicals, etc. from the synthesizing extracts tend to cap the naïve particles [21,22]. The physicochemical properties and behavior of the biogenic ENMs can be characterized accurately under idealized conditions, however, when exposed to the environment, these tend to acquire different properties due to interaction with various biotic and abiotic factors [23]. Herein, proteins and other biomolecules interact with surface of ENMs, forming a biomolecular corona that critically affects their biological and technical identities [24]. The bimolecular corona may impacts the in vitro and in vivo applications of ENMs, and therefore the mechanistic understanding of the biophysical forces regulating their interactions with various biological and environmental factors is required to estimate/predict influence of the biogenic ENMs on humans and environment.

ENMs dispersal in abiotic and biotic environment

The ENMs, when applied in agricultural fields, can find their way to the environmental components: soil, water and air. Interactions of bio-corona with different factors such as different electrolytes with monovalent and divalent ions, natural organic matter (NOM) [25], terrestrial and aquatic plants and animal species; present in the natural environment determine the fate of the ENMs transformations such as dissolution, agglomeration, sedimentation, interaction and release of surface moieties within the immediate surrounding [26,27]. This could greatly affect the pathway and extent of emission and consequently the impact on the human and environment. Yet, it is still an unsolved question to accurately determine the relevant
concentrations of applied biogenic ENMs that will be released from one stratum to another at any given time [28]. It becomes difficult to predict the relevant concentration of biogenic ENMs once released. Such a challenge is due to very little data on prevalence of biogenic ENM as a prospective, commercially available agricultural product [29,30]. Several risk assessment approaches have been used to simulate and calculate predicted environmental concentrations (PECs) of chemically synthesized ENMs but there is an unfilled gap about the biogenic ENMs [31-33].

Most of the inferences on transformation and emission of nanomaterials have been drawn by using chemically synthesized ENMs [34-36]. However, only a few experiments have been carried out under natural conditions to analyze the fate of the biogenic ENMs [37]. Research needs to be expedited to identify environmental transportation pathways of biogenic ENMs, along with subsequent evaluation of their behaviour, fate, dissolution, residence time and concentrations within different media – soil, water and air. For this different mass flow models can be developed to predict the concentration of nanomaterials from manufacturing and after application to various compartments of the environment. Simulations may serve as an important area of focus to assess the physical and chemical transformations of ENMs once adapted in agricultural practices.

**ENM Characterization and Transformation**

Risk assessment and life cycle analysis of the ENMs, especially biogenic ENMs, is a growing field and demands a comprehensive understanding of ENM characterization and transformation. A significant contribution on transformation and fate of environmentally released ENMs is made by studying complex interaction of ENMs with different model systems and mesocosm studies, but several challenges harbor the strategies to study ENM safety [38,39]. Many of these challenges center on the tension between understanding the mechanism of interaction or the ENM transformation as it relates to more complex whole organism or ecosystem models [40,41]. The complexity enhances when there is an intervention of biogenic ENM having an additional bio-corona [42] (Figure 2).

The most common techniques used to characterize ENMs are X-ray based (diffraction, absorption spectroscopy and photoelectron spectroscopy, energy-dispersive X-ray spectroscopy), electron microscopy (transmission and scanning), atomic force microscopy, spectroscopy (UV-vis, infrared, atomic absorption and nuclear magnetic), zeta potential and dynamic light scattering measurements, etc [43]. However, these provide only a static picture of the ENM without consideration of the relevant biological environment [44] and interaction of different biological macromolecules present in bio-media and bio-corona. Such characterization is either performed where the biological environment is ignored, in water or organic solvent alone or under vacuum, or in oversimplified models systems, such as adding BSA to model protein coverage [44]. As the available techniques are insufficient to characterize the ENMs all before, during and after the applications in agricultural fields [45,46], new characterization methods are needed to be studied. These novel techniques can then may be exploited to understand translocation and transfer of the biogenic ENMs protein adsorption (i.e. opsonisation) on the surface of the particle or particle aggregation/agglomeration that may impact the uptake/clearance mechanisms [28,44].

Molecular toxicology provides a basis for much of the nano-safety assessments for all sorts of nanomaterials and their bulk counterparts. However, in relation to risk of cytotoxicity posed by a biogenic ENM as compared to its bulk or chemically synthesized ENM counterparts,
one distinguishing feature of biogenic ENM is the difference in their physical characteristics, including size, crystallinity and importance of surface charge and surface chemistry. When brought in contact with the environment, the ENMs irrespective of their inherent physical nature undergo a transformation. This is attributed to high surface area and thus high reactivity of ENMs towards various molecules resulting in heterogeneous surface composition over them [47,48]. ENM-transformations are the result of a myriad of reactions or processes, including aggregation/agglomeration, redox reactions, dissolution, and reactions with naturally occurring macromolecules and biomolecules. These dynamic transformations in turn affect the transport, fate, and toxicity of ENMs in the body or environment, and therefore, it is critical to understand and characterize these transformations. Typically, physiological or environmental conditions are simulated in the laboratory by modeling ionic strength and protein or NOM content, which are also the key players in ENM transformation in natural environment, particularly soil and water bodies [44].

In these cases, the size of ENMs is an important determinant to reactivity, transport, and toxicity, and while the primary particle size is always estimated (with electron microscopy); ENMs tend to agglomerate in different solutions and biological media which leads to an interaction between a biological system and the aggregate sized material instead of the nanomaterial. Dynamic light scattering techniques are most commonly employed to study stability of ENMs in solution [49] or as an aerosol [50]. With respect to aggregation, the presence of bio-macromolecules present on the surface of ENM and also in media may have varied effects [51]. In the presence of physiological factors like varying pH and salinity, the biomolecules behave in a dissimilar manner and undergo several biophysical alterations which perturbs their native assembly and may result is agglomerative behavior [25]. Therefore, the capped/biogenic ENMs respond differently to the exposed environment as compared to uncapped ENMs [51].

Understanding aggregation is critical for characterizing transport of biogenic ENMs through the body of human and other model organisms and environmental compartments. In the body, greater aggregation yields larger particles that are cleared from the body by the mononuclear phagocyte system [52], and in the environment, less aggregation yields lower rates of sedimentation and greater mobility [44]. Therefore, understanding the interaction of ENMs under natural conditions (e.g. salinities, pH, and molecular species) may enable a better assessment of exposure and transport. These aggregation studies bring to light the importance of the ENM surface and localized environment around that surface in the transformation of the material [36,53,54]. Proteins and other biomolecules like fatty acids can act upon the biogenic ENM leading to free energy change of the surface and/or can influence other ENM transformations [55].

Other kind of ENMs (e.g. chemically synthesized metallic ENMs, such as, formulations of Au, Ag, Ti, Fe, Zn and Cu [56]) experience a similar speciation either in the dissolution to ions or chemical reactions that, in turn, could affect other physicochemical changes associated with them and ultimately their translocation and toxicity. The interplay between the different, dynamic forms of ENMs that may be acquired after interaction with the environmental factors discussed above and the importance of their state on subsequent transport and toxicity necessitates careful time-dependent characterization of biogenic ENMs in environmentally relevant conditions [28].

Considerations of Model Systems

For understanding a complete functional impact of biogenic ENMs on humans and environment, choice of relevant model system and the investigation with them is important. Increasing the complexity of a model system makes the risk assessment difficult and demands the need for the development of advanced methods to address this challenge. In particular, it becomes the necessity of the hour to develop a toxicological methodology with specific in vitro techniques and models' systems that are high-throughput, reproducible (both intra and inter lab), close to mimic human system, as well as predictive of toxic response in vivo.

In the realm of biological model systems, complex culture systems allow for more reliable results and are worthy of further exploration. Common biological and ecological model systems are discussed below:
Biological Models

Biological studies often utilize two categories of model systems: in vivo wherein a whole organism is used, and in vitro, with cells derived from live tissues/ organs or immortal cell lines.

For in vitro studies, there is a wide range in model systems. Choices must be made when selecting a model cell - between use of primary culture or immortal cell lines (i.e. cells isolated directly from an animal or a self-propagating cell line) and use of cells of human origin or animal origin. Also an assessment needs to be made to see if the physiological function of the model cell is relevant, and whether the model cell is found in the tissue likely exposed to the biogenic ENM [43]. Ideally to study human toxicity, primary culture of human cells are the best suitable, but these models are generally restricted to commercially available cells capable of continuous propagation or cell types isolated from small samples of donor blood such as mononuclear cells [57], B lymphocytes, and T lymphocytes [58]. An alternative is to use primary culture of cells from animal models that require less regulation [59]. The major advantages of using the primary cells are their functional and genetic fidelity corresponding to systemic research. Such organ specific primary cells can be derived from the relevant animal models to understand the relevance of systemic exposure to ENMs. Culturing of cells from live model organisms yields a heterogeneous mixture of cells that then typically require density gradient or flow cytometry sorting to isolate the model cell of interest. These separation techniques often damage cells in the process of isolation. Additionally, primary culture cells are often obtained in limited numbers and have a limited lifetime in culture [60]. Due to these key disadvantages, cell lines are commonly used for in vitro testing. A plethora of immortal cell lines is commercially available for use as both cancer cell models and immortalized representations of normal cells. The major drawback of using immortal cell lines is that the mutations required for the immortalization of the lines may affect the way the cells respond to ENMs [61,62]. To improve the reliability of interpretations from immortal models, studies can be compared using different cell lines that have the same physiological function. An alternative method can be the use of co-culture models to yield a better representation of in vivo conditions [63,64], although the presence of two cells in culture may lead to cross contamination and thus, can complicate interpretation.

In vivo studies provide vital information to assess the health and safety aspects of biogenic ENMs as they are capable to demonstrate a systemic response once the model organism is exposed to biogenic ENMs [65]. Depending on the potential application of the ENM, various animal models or physiological mechanisms can be studied, such as zebrafish embryo development [66], rabbit ocular toxicity [67], rat pulmonary toxicity [68] or immune cell distributions in mice [43]. These types of studies have several limitations including the long experimental time required, the high cost, and the ethical concerns regarding the treatment of laboratory animals [43]. Yet, in vivo studies are necessary to explore ENM bio-distribution and determine appropriate cell types to be used in in vitro studies.

In vitro methods are relatively faster, inexpensive and minimize ethical concerns as compared to in vivo studies; however, many of these methods require more extensive validation with in vivo studies to evaluate their toxicological predictive capability and reproducibility.

Ecological Models

Ecological model organisms range from single celled microorganisms to plants and higher order animals. The nano-eco-toxicity generally follows a hierarchical study and thus, choses typical model system based on the strata of food web. Microorganisms form the lower most strata of a typical food chain and are omnipresent in the different ecosystems. Besides their ubiquitous presence, it is evident since long that they play important roles in nutrient cycling. Nano-safety studies have included commonly used research species such as Escherichia coli [69,70], Bacillus subtilis [71] and Pseudomonas aeruginosa, [72] Nitrosomonas europeana [73]. The breadth of choices in these monoculture systems has led to some challenges within the field in generalizing experimental results. That is, the deepest studies utilize the common microbial species that may not be environmentally relevant, while more environmentally relevant species have been considered less thoroughly. To overcome this issue, some research groups have pursued toxicity studies on naturally sampled bacteria [74-76].

Although the research involving micro-organisms give an insight of impact of ENM on biotic environment, a detailed inference can be made only when plant and higher order organism are studied for the effects of ENMs on them.

For understanding the implication of agriculturally useful biogenic ENMs and to define their eco-nano-toxicity plants play a relevant candidate role. Plants have a ubiquitous interaction with ENM contained abiotic components viz. air, water and soil. Additionally, they have a critical role to play in inter-species transfer and bio-distribution of ENMs attributed to their consumption by organisms at all the hierarchical levels of food chain. Notably, most of the nano-safety work related to ENM for agriculture has focused on edible plants (pumpkin, radish and cucumber) and crops (maize, wheat, soybean, tobacco and rice) [77,78].

The ecosystems functions in a regulated manner with a number of aquatic and terrestrial animals. These make to the major components in the food web as food sources. An in-depth comprehension of the impact of biogenic ENMs can be directly related to human health. The Organization for Economic Co-operation and Development (OECD) defines guidelines that consider Japanese medaka [79] and zebrafish as standard organisms for aquatic toxicity testing [80,81]. The ability to quickly reproduce and having a completely sequenced genome, makes zebrafish (a freshwater fish), [82] and medaka (saline habitats) important for understanding genetic impacts of biogenic ENMs in the environment [83].

Conclusion

Globally, an exponential release in nano-enabled product development has been observed since 2000s [84], with a projected growth to over half a million tons by 2020 [85,86]. Agriculture being the prime area to fulfill the global food requirement will entail the applications of next generation nanotechnology [16]. This makes it certain that there has been and will be a continuous release of ENMs in the environment, which will lead to significant human exposure. As an inference, it can be deduced that a thorough understanding on impact of ENM exposure is needed to assess the biological and ecological implications – both beneficial and harmful. It becomes critical within the field of nano-safety to develop specific technology that can enable the understanding of impact of biogenic ENM on a mechanistic level. Development of methods for clear elucidation of the kind of bio-interaction and the effect thereof in various biotic and abiotic components of the environment related to biogenic ENMs is needed. While it can be assessed that the biological and ecological toxicology studies have different aspects, the challenges that are faced by the nano-safety community demands interdisciplinary efforts to
overcome them. However, the dose dependent response should be the preliminary factor to be considered while commenting on the toxic effects of biogenic ENMs, as required dose of such ENMs in agriculture fields could be much lesser as compared to the bulk materials. Also due to virtue of transfer and transformation across the different trophic levels in the environment, there are chances that the biogenic ENM concentration may get reduced to an inconsequential amount, thereby decreasing the probability of an ill-effect to infinitesimal.

References

1. Aiken GR, Hsu-Kim H, Ryan, JN (2011) Influence of dissolved organic matter on the environmental fate of metals, nanoparticles, and colloids. *Environ Sci Technol* 45: 3196-3201.
2. Amde M, Liu JF, Tan ZQ, Bekana D (2017) Transformation and bioavailability of metal oxide nanoparticles in aquatic and terrestrial environments: a review. *Environ Pollut* 230: 250-267.
3. Arias-Estévez M, López-Péreiro E, Martínez-Carballelo E, Simal-Gándara J, Mejuto JC, et al. (2008) The mobility and degradation of pesticides in soils and the pollution of groundwater resources. *Agriculture, ecosystems & environment* 123: 247-260.
4. Arnaout CL, Gansch CK. (2012) Impacts of silver nanoparticle coating on the nitration potential of Nitrosomonas europaea. *Environ Sci Technol* 46: 5387-5395.
5. Barbero-CA, Yalas EI (2017) Ecotoxicity effects of nanomaterials on aquatic organisms: nanotoxicology of materials on aquatic organisms. Applying nanotechnology for environmental sustainability 330-351.
6. Batley GE, Kirby JK, McLaughlin MJ (2012) Fate and risks of nanomaterials in aquatic and terrestrial environments. *Acc Chem Res* 46: 854-862.
7. Beloin-Saint-Pierre D, Turner DA, Salieri B, Haarman A, Hischier R (2018) How suitable is LCA for nanotechnology assessment? Overview of current methodological pitfalls and potential solutions: 65th LCA Discussion Forum, Swiss Federal Institute of Technology, Zürich, May 24, 2017. *Int J life cycle Asses* 22: 191-196.
8. Brayner R, Ferrari-Iliou R, Brivois N, Djediat S, Benedetti MF, et al. (2006) Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium. *Nano letts* 6: 866-870.
9. Bruckner S, Rhamouni S, Tautz L, Denault JB, Alonso A, et al. (2005). Versinia phosphatase induces mitochondrial-dependent apoptosis of T cells. *J biological Chem* 280: 10388-10394.
10. Chaudhry ND, Dwivedi S, Chaudhry V, Singh A, Saquib Q, et al. (2018) Bio-inspired nanomaterials in agriculture and food: Current status, foreseen applications and challenges. *Microbial pathogen* 123: 196-200.
11. Christian P, Von der Kammer F, Baalousha M, Hofmann T (2008) Nanoparticles: structure, properties, preparation and behaviour in environmental media. *Ecotoxicology* 17: 326-343.
12. Conley DJ, Paerl HW, Howarth RW, Boesch DF, Seitzinger SP, et al. (2009) Controlling eutrophication: nitrogen and phosphorus. *Science* 323: 1014-1015.
13. Das P, Williams C, Fulthorpe RR, Hoque ME, Metcalfe CD, et al. (2012) Changes in bacterial community structure after exposure to silver nanoparticles in natural waters. *Environ Sci Technol* 46: 9120-9128.
14. Douglas Gellin DL, Waugh M, Saced S (2012) The Agricultural Productivity Gap in Developing Countries. *International Growth Center*.
15. Dransfield I, Bickle A, Savill JS, McDowall A, Haslett C, et al. (1994) Neutrophil apoptosis is associated with a reduction in CD16 (Fc gamma RIII) expression. *Journal of immunology* 152: 198-200.
16. Dumortier H, Lacotte S, Pastorin G, Marega R, Wu W, et al. (2006) Functionalized carbon nanotubes are non-cytotoxic and preserve the functionality of primary immune cells. *Nano letts* 6: 1522-1528.
17. Fischer HC, Chan WC (2007) Nanotoxicity: the growing need for in vivo study. *Curr Opin Biotechnol* 18: 565-571.
18. Fresta M, Fontana G, Bucalo C, Cavallaro G, Giannona G, et al. (2001) Ocular tolerability and in vivo bioavailability of poly (ethylene glycol) (PEG)-coated polyethyl-2-cyanoacrylate nanosphere-encapsulated acyclovir. *J Pharm Sci* 90: 288-297.
19. Furutani-Seiki M, Wibbrot J (2004) Medaka and zebrafish, an evolutionary twin study. *Mechanisms of Development* 121: 629-637.
20. Gavankar S, Suh S, Keller AF (2012) Life cycle assessment at nanoscale: review and recommendations. *International journal of life cycle assessment* 17: 295-303.
21. Ge Y, Schimmel JP, Holden PA (2011) Evidence for negative effects of TiO2 and ZnO nanoparticles on soil bacterial communities. *Environ Sci Technol* 45: 1659-1664.
22. Godfrey HJC, Beddington JR, Grice ER, Haddad L, Lawrence D, et al. (2010) Food security: the challenge of feeding 9 billion people. *Science* 1185383.
23. Gollin D, Lagakos D, Waugh M. (2011) The agricultural productivity gap in developing countries. *Unpublished manuscript*.
24. Gordon S (2016) Phagocytosis: an immunobiologic process. *Immunity* 44: 463-475.
25. Gottschalk F, Scholz RW, Nowack B (2010) Probabilistic material flow modeling for assessing the environmental exposure to compounds: Methodology and an application to engineered nano-TiO2 particles. *Environ modelling & software* 25: 320-332.
26. Guo Z, Cui K, Zeng G, Wang J, Guo X (2018) Silver nanoparticles in the natural environment: An overview of their biosynthesis and kinetic behavior. *Sci Total Environ* 643: 1325-1336.
27. Gupta H (2018) Role of nanocomposites in agriculture. *Nano hybrids and composites* 81-89.
28. Hase A, Lynch I (2018) Quality in Nanosafety-Towards a reliable nanomaterial safety assessment. *Nanomaterial* 11: 67-68.
29. Hayami Y (1969) Sources of agricultural productivity gap among selected countries. *Am J Agricultural Econ* 51: 564-575.
30. He X, Aker WG, Lesczynski J, Hwang HM (2014) Using a holistic approach to assess the impact of engineered nanomaterials inducing toxicity in aquatic systems. *J Drug Anal Lett* 22: 128-146.
31. Hischier R, Walser T (2012) Life cycle assessment of engineered nanomaterials: state of the art and strategies to overcome existing gaps. *Sci Total Environ* 425: 271-282.
32. Hu X, Sun A, Kang W, Zhou Q (2017) Strategies and knowledge gaps for improving nanomaterial biocompatibility. *Environ Int* 102: 177-189.
33. Huang F, Gilbert B, Zhang H, Banfield JF (2004) Reversible, surface-controlled structure transformation in nanoparticles induced by an aggregation state. *Phys Rev Lett* 92: 155501.
34. Ingham RI, Troyfymow J, Ingham ER, Coleman DC (1985) Interactions of bacteria, fungi, and their nematode grazers: effects on nutrient cycling and plant growth. *Ecological monograph* 55: 119-140.
35. Justino CL, Rocha-Santos TA, Duarte AC (2011) Sampling and characterization of nanoaerosols in different environments. *Trends in Anal Chem* 36: 554-567.
36. Kab M, Kookana RS, Gogos A, Bucheli TD (2018) A critical evaluation of nanoparticid and nanofertilizers against their conventional analogues. *Nat Nanotechnol* 13: 677-684.
37. Kasahara M, Naruse K, Sasaki S, Nakatani Y, Wu W, et al. (2007). The medaka draft genome and insights into vertebrate genome evolution. *Mechanisms of Development* 123: 1452-1453.
38. Khan R, Inam MA, Zam SZ, Park DR, Yeom IT (2018) Assessment of key environmental factors influencing the sedimentation and aggregation behavior of zinc oxide nanoparticles in aquatic environment. *Water* 10: 660.
39. Khati P, Gangola S, Bhatt P, Kumar R, Sharma A (2018) Application of nanocompounds for sustainable agriculture system. *Microbial biotechnol Environ monitoring and cleanup* 194-211.
40. Klaine S, Alvarez P, Batley G, Fernandes T, Handy R, et al. (2008) Critical review of nanomaterials in the environment. *Environ Toxicol Chem* 27: 1825-1851.
41. Lee JK, Nallathamby PD, Browning LM, Osgood C, Xu XH (2007) In vivo imaging of transport and biocompatibility of single silver nanoparticles in early development of zebrafish embryos. *ACS Nano* 1: 133-143.
42. Lei C, Sun Y, Tsang DCW, Lin D (2018) Environmental transformations and ecological effects of iron-based nanoparticles. *Environ Pollut* 232: 10-30.
43. Levard C, Hotze EM, Lowry GV, Brown GE (2012) Environmental transformations of nanoaerosols in different environments. *Int J life cycle Asses* 17: 295-303.
