Chapter 6

Summary

Although the use nanomaterials are going on for quite few years, the environmental fates of purposefully synthesized nanoscale materials are drawing greater attention recently because of their widespread use. Numerous metal based nanomaterials are used in various applications in the fields of information technology, electronics, catalysis, medicine, and energy owing to their electronic, thermal, optical, and photoactive properties. As such, synthesized vis-à-vis engineered metal based nanoparticles are widely used in consumer goods and industrial processes. Consequently, the invisible release of engineered nanomaterials to the environmental matrix is significantly increasing; thereby leading to long range contamination and/or alteration of the natural resources of our planet. Soils being the ultimate sink of all terrestrial biotic and abiotic substances, gradual piling up of nanoscale metal based materials in soils is inevitable. To date, there is very little understanding on how such discharged nanomaterials influences the soil physicochemical properties; therefore research reports were not adequate at the beginning of this study to render a guideline for formulating the experimental designs. Overall, it was clear that silver and metaloxide nanomaterials are the most predominant among various types of synthesized nanomaterials. Silver nanoparticles (AgNPs) have been widely used in cosmetic products and as components of antimicrobial drugs as compared to other forms. In recent past, the promising role of AgNPs as effective and alternative pesticides to the conventionally applied synthetic organic compounds has been identified. On the other hand, the use of metal oxide nanomaterials is expanding; their collateral exposure to the soil and water environment is on the rise. Contrastingly, metal oxide nanomaterials have greater prospect to cater the micronutrient deficiency in agriculture.

This research was conducted with two broad aims: firstly, to study the impacts of AgNPs on soil plant continuum with special references to soil fauna and earthworm was taken as the model to assess such impacts via short and long term experimentations; secondly, to explore the possibility to synthesize efficient iron oxide nanomaterials for application in agriculture as soil conditioners because iron is an essential micronutrient. As such, the impacts of AgNPs and iron oxide nanomaterials greatly vary depending on their synthetic routes; thus proper understandings cannot be developed through short term lab scale experiments. Under these contexts, the AgNP was prepared using plant extracts and the oxalate capping was impregnated to synthesize an iron oxide (Fe_3O_4) nanomaterial after several trials. Then, the synthesized nanomaterials were exposed to both aqueous and soil mediums in laboratory and in ambient conditions. At first, they were applied to soil samples devoid of plants in laboratory condition; then, they were applied to crop plants grown in potted soils as well as in farmer's field.

The chapter 1 broadly describes the background and basis of this research in the form of introduction and also identified the relevant research questions and hypotheses; on the basis of that the research objectives were formulated. The second chapter of this thesis has described an extensive review of relevant literatures which has been followed by the overall plan of the research adopted to address the identified research objectives in chapter 3. The main focus of this research was on the behavior of silver and iron oxide nanomaterials in soil-plant interfaces. Therefore, the detailed descriptions of all the experiments and their results (synthesis, characteristics, short and long term impacts on soil health and plant growth) for silver and iron oxide nanomaterials have been separately presented in chapter 4 and chapter 5 respectively.

The experiments for silver nanoparticles have been conducted in a phased manner (chapter 4). Initially, it was difficult to formulate a reliable as well as comprehensive experimental plan to address the impacts of AgNPs on soil and plant health because of large variations among the experimental results of the previous researchers. In fact, in this endeavor the outcomes of the same nanoparticles considerably varied. Two types of silver nanoparticles were used in the short term study. At the beginning the silver nanoparticles were synthesized following previously standardized synthetic routes; however, one of such routes was considered as 'green' because the sodium borohydride was replaced by the leaf extracts of *Thuja occidentalis* for reducing the Ag^+ to Ag^0 in the nano form (size 7-14 nm). Whereas, another SNP i.e., conventional SNP (CSNP) was prepared using sodium borohydride. Various doses of CSNP and GSNP were applied to potted soil samples and French bean was grown in such plots. In addition, the stress of GSNP exposure was assessed in tomato. The soil of this experiment was a typical inceptisol at an early to medium stage of soil formation. The impact of GSNP on soil fertility status was encouraging while, the growth of N-fixing and P-solubilizing bacteria was noticeably augmented due to GSNP (25 and 50 ppm) exposure on soil under French bean cultivation. The experiment also indicated that the

GSNP might have played some role in inhibiting the leaching dynamics of NO_3-N in soil. This particular hypothesis was verified through batch scale experiments and it could have been concluded that the nitrate-N in the soil was held in the solid matrix against the gravitational water pool may be either by the additional adhesive force created due to presence of Ag^0 in the soil or due to generation of greater anion exchange sites owing to the conversion of Ag^0 to Ag^+ in the soil system. Correspondingly, the growth and development of GSNP treated French bean was significant along with the evidence of upregulation of nitrate reductase and ferredoxin responsive enzymes and their respective genes. On the other hand, the N assimilation in AgNP treated plant was significantly retarded probably due to down regulation of glutamine synthetase (GS) and glutamate synthase (GOGAT) enzymes along with their respective genes. Moreover, the rate of photosynthesis and chlorophyll content were substantially jeopardized in AgNP exposed tomato. The evidences of oxidative stress in regard to elevation in lipid peroxidation, proline content, catalase activity in AgNP exposed plant were also prominent.

Although, the overall results of this study were surprisingly beneficial to soil health, the impacts on plants varied significantly depending on the plants species used as the model. Hence, it was presumed that the apparent beneficial role of the GSNP (hereafter AgNP) was not translated uniformly at plant level. Therefore, it was necessary to plan a long term (72 weeks) and in depth research to appreciate the behavior of the AgNP in soil system. The impact of the *Thuja* mediated AgNP was studied in not only soil and aqueous media but also assessed in earthworm models because earthworms are vital indicators of soil health and thus are authentic objects for eco-toxicity assessments. The probable mechanisms of the apparent changes in soil properties were ascertained through lab scale batch experiments in aqueous media. The interesting outcomes of the experiment suggested that the effects of AgNP on soil quality were largely dependent on the concentration and duration of the exposure. Such retardation was greater with higher concentration (25 and 50 mg kg^{-1}) than the lower concentrations and the DLS and UV-VIS based monitoring suggested that aggregation of AgNP was greater up to 8-12 weeks from the date of exposure. In contrast, the dissolution of aggregated nanoparticles might have increased with further ageing which in turn greatly affected availability of nutrient elements (N, P, K, and S). Similarly, earthworm growth and fecundity was normal up to 50 ppm level of exposure but the expression of oxidative stress indicating enzymes (catalase, glutathione reductase etc.) were greatly upregulated. In addition, the histological analysis revealed the chloragogenous tissue layers of the earthworms were severely damaged due to AgNP exposure.

One of the major challenges of this research was to utilize the uniqueness of the nano scale iron oxide materials for agricultural benefit in an ecofriendly manner. Fortunately, a novel oxalate capped iron oxide nanomaterial (OCIO) would have been synthesized after several trials and eliminations (chapter 5). The synthetic route was easy, sustainable, and scalable to meet up the large scale demand in agriculture. The moderate (10 ppm) exposure of the OCIO greatly improved seed germination and they were harmless to beneficial soil bacteria. Moreover, oxidative stress in plants was negligible upon 10 ppm exposure levels of the nanomaterial. Fascinatingly, the material provided improved soil conditioning by i) balancing pH; ii) sustaining Fe, Cu, and Mn availability without increasing soil acidity thereby promoting P release; iii) facilitating availability of N, and K; iv) reducing bulk density; and v) mobilizing soil microbes and enzyme activity owing to their porous morphology, small size, high solubility, and excellent buffering capacity. As such, the underlying mechanisms of the prolific impacts have been determined through miniature experimentations. On-field crop experiment revealed that OCIO corrected micronutrient deficiency in soil and significantly augmented tomato growth. The growth inducing mechanisms in plants could be the greater nutrient uptake due to profound root growth, favorable rhizospheric ambience (stabilized pH, high porosity, and microbial proliferation); greater N-assimilation; upregulation of vital genes responsible for root growth, photosynthesis, and N-assimilation (*RSL4*, *MATE8*, *Ferredoxin*, *GS2*, *GOGAT*, and *NR*); and adequate development of photosynthetic apparatus leading to greater photosynthesis as compared to conventionally used $FeSO_4$ and Fe-EDTA chelates. Overall, the OCIO application in arable soil augmented crop production by 3-4 folds with 8-10 folds lower dose than traditional micronutrient fertilizers.