ABSTRACT

Metal based engineered nanomaterials are abundantly found in nature because of their wide application in commercial product development processes. The focal point of the present endeavor was to study the behaviour of metal based engineered nanomaterials in soil–plant systems. For the purpose of the study, silver and FeO nanomaterials were selected. Silver is a non-essential element, while it's use in nanotechnology is widespread. On the other ground, iron is a vital plant-derived essential micronutrient in the human diet. Fe availability in soil largely depends on the pH and leaching behaviour of the soil. Although common salts ($FeSO_4$) and chelates (EDTA) of Fe ensure high availability of the nutrient, they often interfere with P availability in the soil. Considering such disadvantages of the well-known Fe sources, an attempt was made to evolve efficient Fe_3O_4 nanomaterials that can be used in agriculture as soil conditioner.

In this research, the silver nanoparticle was synthesized following a standard methodology published earlier. The impacts of silver nanoparticles have been reported to be detrimental for the environment; however, such impacts have rarely been thoroughly studied in soil plant continuum. Hence, a previously standardized protocol was followed to prepare the Ag nanoparticles using leaf extracts of *Thuja occidentalis*; the physico-chemical properties of this nanoparticle have been thoroughly studied earlier and that information were useful to interpret the results of our experiments. On the other hand, an effort was made to synthesize an oxalate capped Fe_3O_4 nanomaterial on large scale basis for agricultural application. The major pre requisites for determining the synthetic route were: a) yield per reaction should be higher (at least in kg scale); b) such nanomaterial should not be toxic to soil biota at least to a certain level of concentration; c) nutrient content (Fe) and release profile should be high and efficient respectively; d) the nanomaterial should be mostly soluble within a short period of time after application; e) the nanomaterial application should not retard bio availability of other nutrients and must not induce drastic alteration in the soil reaction. Fortunately a novel synthetic route could have been developed and the end product fairly meeting all the pre requites. The synthetic routes of the nanomaterials have been discussed in chapter 3.

Prior to the materials and method section the detailed background of the study and importance of the recognized problems were thoroughly discussed in chapter 1 (General

introduction). The literature review is comprehensively discussed in the chapter 2 of the thesis. The chapter 3 comprised of the general methodology and overall research plan of the study.

Impact of synthesized nanomaterials on soil health: This objective has been dealt in chapter 4 and 5 of the thesis. The efficacy of AgNP on soil quality was systematically described in chapter 4. Initially, a short term soil incubation study was conducted with the concentrations ranging from 20-100 mg kg^{-1} (20, 25, 50, and 100) to identify the environmental feasibility of the green silver nanoparticles (GSNPs or AgNP) was assessed in regard to their effects on soil physicochemical properties and plant growth in comparison with conventionally synthesized silver nanoparticles (CSNPs). Upon application of GSNPs, soil pH shifted toward neutrality, and substantial improvements were observed in water holding capacity (WHC), cation exchange capacity (CEC), and nutrient (N and P) availability. However, retardation in nutrient availability and enzyme activity was apparent in soils treated with 100 mg kg^{-1} of either CSNPs or GSNPs. According to many researchers short term pot culture experimentation might not be authentic to appreciate the AgNP impacts on soil environment on longer perspective. Therefore, on the basis of the significant outcome of the short term experiment a long term and inclusive study encompassing soil, plants, and earthworms was conducted to identify the AgNP behaviour in soil system through a long term (72 weeks) soil experiment. The 50 ppm exposure of AgNP greatly retarded nutrient availability and microbial growth in soil after 12 weeks of exposure. Contrary to previous reports, we demonstrated that dissolution rate of AgNP increased with time in soil. Dynamic Light Scattering and UV-VIS assessments exhibited concentration and time dependent agglomeration of AgNP in soil media.

Noteworthy outcomes were recorded in 90 days long soil exposure of OCIO. Significantly higher P availability was recorded in soil media treated with OCIO as compared to $FeSO_4$ and Fe–EDTA. Additionally, application of OCIO@10– 20 mg kg^{-1} considerably increased organic C, N, P, and enzyme activity (urease and phosphatase) in soil. In addition, the OCIO remarkably recovered Fe deficiency in soil, maintained steady P availability, and stabilized pH of the poorly fertile soil, promoted healthy growth and productivity of tomato. Concurrently OCIO application among various doses, 10 mg kg^{-1} was found to be efficient for OCIO as compared $FeSO_4$ (50 mg kg^{-1}) and Fe-EDTA (10 mg kg^{-1}).

Impact of nanomaterials on leaching and solubility patterns of ions in soil and aqueous media:

Chapter 4 describes the impact of AgNP on leaching and solubility patterns of ions in soil and aqueous medium; while the chapter 5 deals with solubility patterns of ions in the OCIO inoculated soil and aqueous media.

A focused column experiment indicated that the AgNP abundance in soil might have reduced nitrate leaching, thereby sustaining N availability in root zone soil layers. Additionally, a lab scale batch experiment confirmed greater N contents in AgNP treated sterilized soil than the control. However, in both aqueous and soil medium AgNP incorporation sharply acidified the conditions. Moreover, lab based experiments in aqueous medium revealed that significant reduction in silver availability after 12 weeks of exposure was due to formation of Ag_2S or Ag_3PO_4; which also greatly affected the P and S availability.

In chapter 5 it is highlighted that the Fe release profile from OCIO was uniform at all pH (4-9). The OCIO material was not only novel in regard to its synthetic route but also it shows a unique buffering capacity which is generally very rare in such kind of nanomaterials. Correspondingly this specific quality also has had great beneficial impact in agricultural soil. The lab based batch experimentations were conducted to realize the underlying mechanisms of such excellent buffering capacity of OCIO. It was apprehended that the polymeric framework of the nonmaterial could able to scavenge the excess H^+ under acidic conditions. In fact, the Fe-oxalate might also have played a role of proton donor under alkaline conditions.

Impact of nanomaterials on crop responses:

One of the major emphases of this study was to understand the applicability of the synthesized nanomaterials in agriculture. The dose dependent effect of AgNP and OCIO were elaborately studied through both short term (pot culture) and long term (field based) experiments. The short term pot culture experiment was conducted keeping French bean (*Phaseolus vulgaris*) and tomato (*Lycopersicon esculentum*) as the test crops for silver nanoparticles. Although plant experimentation in tomato based models is common in the literature, the impact of AgNP on French bean was conducted to assess the crop-dependent response. Remarkable improvements in leaf area index

(LAI), leaf number, chlorophyll content, nitrate reductase (NR) activity, and *P. vulgaris* pod yield were observed after the application of low doses of GSNPs (25–50 mg kg^{-1}). The true benefit of GSNP application to soil was substantiated through experiments on plant uptake of nutrients, *NR* gene, and ferredoxin (*Fd*) gene expression in *P. vulgaris* leaves. In contrast, 10 mg kg^{-1} exposure of AgNP sharply induced oxidative stress in tomato; which was strongly correlated with Ag uptake in plants. Such exposure of AgNP also retarded activity of N-assimilating enzymes (glutamate synthase, glutamine synthetase, and nitrate reductase) by suppressing their genes (*GS2* and *GOGAT*) in tomato. Eventually, photosynthesis and CO_2 assimilating efficiency were severely disrupted; and finally overall yield of the crop (tomato) was severely reduced as compared to untreated plants.

The outcome of the pot culture studies with AgNP was confusing because both useful and harmful effects of AgNP were observed on plants. Therefore, a 2 year long field experiments were conducted on farmer's field in a typical new alluvial soil zone of Assam. However, the impact of AgNP was not encouraging. Significant reduction in crop yield, and storage quality (Shelf life) of tomato were recorded in AgNP treated plots as compared to the control.

However, the impacts of OCIO on plant growth and metabolism were highly encouraging in both miniature (pot-culture) and large scale field experiments. Overall, considerable yield advantage in tomato was achieved in deficient potted soils as well as in field condition. OCIO application increased chlorophyll activity, photosynthetic rate, shelf life, and lycopene content in OCIO treated tomato as compared to untreated, $FeSO_4$, and Fe-EDTA in field condition. Remarkable increment in fruit yield along with, enhancement in, chlorophyll content was noticed in OCIO treated tomato in pot study with nutrient deficient soil. Subsequently on application of OCIO, oxidative stress indicators were also found in very low amount. Moreover, significant upregulation of the activity of enzymes related to N-assimilation (glutamine synthetase, glutamate synthase, and nitrate reductase) and their respective genes (*GS2*, *GOGAT*, *NR*, and *Fd*) were noticed under OCIO treated tomato.

Impact of nanomaterials on health and reproduction of soil borne earthworms:

The eco toxicity studies for nanoparticles are widely conducted in many animal models. In this research, the focus was on soil; therefore, earthworm model was regarded as adequate on the basis of the available literature. The impact of AgNP on health and reproduction of *Eisenia fetida* was thoroughly studied for a period of about 4 months. The selected concentrations for this eco-toxicity study were 10, 25, and 50 mg kg^{-1}. AgNP exposed earthworms did not show reproductive failure; yet high oxidative stress (catalyse, GST, GSH, and GPx) and reduced protein synthesis led to significant weight loss. Such stress was highest with $AgNP_{50}$ exposure. Subsequently, histological study revealed distortion in chloragogenous tissues (CT) layers in the intestinal lumens of the earthworm body.

On the other hand, there was no detrimental effect on earthworm fecundity and lifecycle due to exposure of OCIO. Moreover, significantly high growth and fecundity rate was noticed along with reduction in oxidative stress under OCIO exposed earthworms. Interestingly, the 10 mg kg^{-1} concentration of the OCIO exposure increased the growth and fecundity of *E. fetida*. This result vindicated the eco-friendly character of this novel nanomaterial when applied in lesser amounts. The details of the findings of this study have been furnished in chapter 4 (AgNP) and 5 (OCIO) of the thesis.

Hence, we can conclude that the AgNP synthesized through green routes showed apparent beneficial impacts on soil and plant systems as compared to the control in the short run. However, in the long term experiments, soil fertility reduces (reduction in pH, nutrient availability, and microbial status in soil) due to AgNP exposure after attainment of some incubation period. Crop growth and yield was also effected due to poor fertility of soil in long term study. Whether, application of OCIO in arable soil significantly enhanced nutrient availability, activities of soil enzymes, augmented microbial growth in both pot culture and on field trials. Remarkable increment in yield of OCIO treated tomato substantiated the potential of this material as a beneficial soil conditioner. Considering the novelty and significance of the outcomes of the OCIO based study the synthesis and application of the material have been applied for patents **(Application Numbers: India – 201631010727; International - PCT/IN2017/050114)**.