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REVIEW

Nanomaterials in Soil Environment: A Review

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ABSTRACT

Nanomaterials (NMs) have become an integral part of our daily life, and their extensive production will only increase with the increasing time. These NMs exhibit significant contrast regarding dimension, reaction, and structure. The most important aspect of the NMs is that these can be easily manipulated and engineered to custom-suit different functions/industries. Owing to their dynamic nature, these NMs behave differently when introduced in any medium. In soil, the behavior of NMs is significantly controlled by the interactions of nanomaterials with soil phases. Although NMs are deemed beneficial for human-use, yet these also carry lethal effects. Moreover, there is a dearth of adequate research for the interactions among nanomaterials and soil physicochemical properties; their accumulation-dissolution dynamics in soil-plant systems; and their long term influence on soil health. Several NMs induce physiological stress when introduced inside the body. Thus, various researchers have devised green pathways for producing NMs, although their wide applicability is still questionable. Although the domain of nanotechnology is greatly explored, yet there remain several grey areas which need to be addressed for sustainable utilization of these unique materials in the benefit of humankind.

1. Nanotechnology and Nanomaterials

Nanotechnology is the branch of science that deals with materials spanning 1-100 nm in size. These materials are produced after altering the physical architecture at the atomic, molecular, and supra-molecular scale [1]. Nanomaterials (NMs) are popularly termed as 0D, 1D, 2D, and 3D based on their physical dimensions [1,2]. Nanoparticles are abundant in nature, say the fragments of colloidal particles (1-1000 nm) which possess at least one dimension in the range 1-100 nm are termed as natural nanoparticles [3]. These natural nanoparticles are formed by geological processes like volcanic eruptions, weathering, microbial action, etc. and can be biological (*e.g.* viral cages, biomolecules, complex carbohydrates, etc.) or inert/non-living in origin *viz.* Al-silicates, O-hydroxides, etc. [4]. On the other hand, artificially produced NMs also known as Engineered NMs (ENM) are significantly different structurally and chemically from their natural counterparts [5]. Recently, great focus has been put on ENMs due to their plethora of applications in the health-care, food, industrial, and environmental sectors [6].

Generally, ENMs bear unique structure and a large

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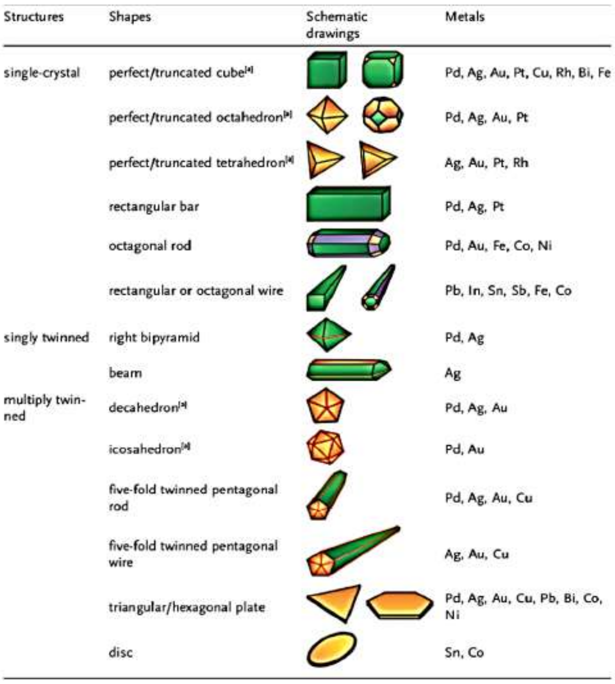
area-to-volume ratio which give rise to the special character and behavioral attributes. These unique features also allow scientists to produce different types of ENMs custom-suited for specific applications [7,8]. Owing to their large scale production several ENMs are now being synthesized on a large-scale. Speaking from a production point of view, ENMs may be either metal based or oxides/oxyhydroxides of different metallic precursors [8].

2. Metal-based ENMs

The NMs of metallic origin are termed as metallic NMs (MNMs) and these are the most widely used NMs owing to their easy synthesis and utility in various industries [9].



Structures	Shapes	Schematic drawings	Metals
single-crystal	perfect/truncated cube[a]		Pd, Ag, Au, Pt, Cu, Rh, Bi, Fe
	perfect/truncated octahedron[b]		Pd, Ag, Au, Pt
	perfect/truncated tetrahedron[a]		Ag, Au, Pt, Rh
	rectangular bar		Pd, Ag, Pt
	octagonal rod		Pd, Au, Fe, Co, Ni
	rectangular or octagonal wire		Pb, In, Sn, Sb, Fe, Co
singly twinned	right bipyramid		Pd, Ag
	beam		Ag
multiply twinned	decahedron[a]		Pd, Ag, Au
	icosahedron[a]		Pd, Au
	five-fold twinned pentagonal rod		Pd, Ag, Au, Cu
	five-fold twinned pentagonal wire		Ag, Au, Cu
	triangular/hexagonal plate		Pd, Ag, Au, Cu, Pb, Bi, Co, Ni
	disc		Sn, Co

Figure 1. A gist of different structural conformations of metallic NMs

Source: Reproduced from Xia et al. [10] with permission (© 2009, John Wiley and Sons, USA).

Also, the metallic precursors of these NMs cover more than two-thirds of the periodic table making them an interesting subject for novel discoveries in material science. Fascinatingly, the metallic NMs exhibit great diversity in their structural properties which not only renders them unique characters but also influences their applicability to a large extent [9]. A summary of various possible structural conformations of the metallic NMs has been provided in Figure 1 [9,10].

In their work, Bratlie et al. [11] elaborated the importance of NM's structure on their catalytic properties. In any catalytic reaction, it is quintessential to attain perfect binding between the reagent molecules and the catalyst (here NM) to form new bonds *i.e.* the product molecules. This condition relies on: (1) the available orbitals of the reagent molecules, and (2) atomic arrangement of the NM surface. Hence, manipulating the structural arrangement of NMs could be a major step towards designing more efficient metallic NMs [9].

3. Metal-oxide NMs

Recently a lot of interest has cropped up on metal-oxide NMs (MONMs) which is mainly credited to their diverse applicability in medicine, agriculture, and engineering [12]. The MONMs exhibit unique physico-chemical properties attributed by their high density and limited corner/edge size on the surface sites. Thus the MONMs duly fill the gap between bulk materials and atomic/molecular structures which attests their importance in nanotechnological applications [13]. In many MONMs *viz*. CuO, ZnO, TiO_2, SnO_2, Al_2O_3, MgO, AgO, CeO_2, ZrO_2 etc. it was observed that reduction in size increased surface strain/stress and concomitant structural perturbations [14-16]. Apart from size and shape attributes, some other important parameters that determine NM properties and application are (i) ionic strength, (ii) zeta potential, and (iii) valency of the metallic precursors in the NMs [17].

(1) Ionic strength: The ionic strength of the electrolyte is an important parameter which determines the behavior of the NMs in solutions. French et al. [18] reported that TiO_2 NMs form stable aggregates at 0.0045 M NaCl solution which changed to micro-aggregates within 15 min when the ionic strength was increased to 0.0165 M. Comparatively, it was noticed that the same NMs (TiO_2) when introduced in 0.0128 M $CaCl_2$ solution form micro-aggregates in 5 min.

(2) Electrical charge and zeta-potential: In general, M NMs bear negatively charged surfaces. This may be provided by the negatively charged hydroxyl ions. Moreover, the high negative surface charge facilitates easy binding of the NMs with moieties. Although, the efficiency of forming bonds is significantly influenced by the inherent reaction (pH), the concentration of the NMs, as well as constituents of the medium [8]. In many MNMs, "double-charged layer" is characteristically present. The double-charged layer is formed by a charged surface layer and a diffused charged layer. The latter is formed by the adhered ions that the NMs attract from the medium. As a result, the difference in the electrical potential originates between the diffused layer and the medium which is termed as zeta-potential.

(3) Valency: The combining power of any NM with other elements to form nano-complexes is influenced

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by the valency. According to Pieters et al. [19], a proper understanding of valency of NMs is of utmost importance to yield efficient catalytic NMs. Similarly, it is also very vital to know about the valence band and the conduction band which modulate the structural properties of NMs [20]. Figure 2 presents a typical 1D band scheme for direct and indirect bandgap semiconducting metal-based NMs where the direction of the wave vector is fixed irrespective of the magnitude.

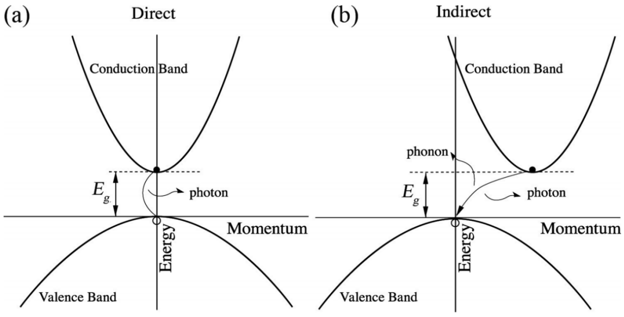

Figure 2. One-dimensional band diagrams containing (a) direct and (b) indirect bandgaps

Source: Martin-Palma and Lakhtakia [20].For further reading please visit: https://www.spiedigitallibrary.org/ebooks/TT/Nanotechnology-A-Crash-Course/3/Properties-of-Nanostructures/10.1117/3.853406.ch3?SSO=1 (© 2010, Society of Photographic Instrumentation Engineers, USA)

Au-NMs are widely used as physical vectors for drug delivery systems and such property is duly induced by the multivalent characteristics of Au nanomaterials [19]. Today, with the advancement in nanotechnology it is possible to produce poly-valent NMs. Such poly-valent NMs have the speciality to change their valency depending on the charge of the target [19].

3.1 Silver NM

Silver is a transition metal with a soft texture and white-lustrous color. It has properties like high electrical and thermal conductivity. Generally, Ag-NMs is very popular due to its anti-microbial activity [21]. Due to their novel properties, the incorporation of silver nanoparticles into different materials like textile fibers and wound dressings can extend their utility on the biomedical field while inhibiting infections and biofilm development [22]. Several workers reported different synthetic pathways for Ag-NM *viz.* physical, chemical, and biological. Evaporation-condensation and laser ablation are the most used physical approaches for Ag-NM synthesis [21]. According to Wiley et al. [23], laser ablation offers pure and clean metallic Ag-NMs without using chemical reagents in solution. Chemical reduction is the mostly used chemical method of Ag-NM production. In this method, several reducing agents *viz*. sodium borohydride, N, N-dimethylformamide, ascorbic acid, hydrazine, etc. are used to reduce Ag^+ in aqueous or nonaqueous solutions [21]. However, due to the contingent toxic nature of these chemical precursors, Ag-NMs are deemed highly toxic for living organisms. In this regard, biologically assisted green synthesis of Ag-NMs has gained a lot of momentum recently. Green synthetic systems use any biological microorganisms such as bacteria, fungi (yeast) and plant extracts for preparing nanoparticles. The interesting review by Pandian et al. [24] elaborated several bio-assisted synthetic methods of silver nanoparticles with a special emphasis on plant-leaf extract mediated synthesis of AgNMs to be an emerging area in the field of nanotechnology.

3.2 Iron oxide NM

Naturally, Iron oxide is found as a mineral with a polymorphic crystalline structure and magnetic properties [25]. The three major polymorphs of Fe-oxide are magnetite (Fe_3O_4), hematite (α-Fe_2O_3), and maghemite (γ-Fe_2O_3). The maghemites mainly in the forms of β-Fe_2O_3 and ε-Fe_2O_3 are generally prepared in laboratory conditions as Fe-oxide NMs [26]. Although several methods are followed for the lab-scale synthesis of Fe-oxide NMs, the most widely used methods are co-precipitation, sol-gel, microemulsion and thermal decomposition methods [26]. The co-precipitation pathway involves dissolving a stoichiometric mixture of Fe (II) and Fe (III) salts in a basic aqueous medium of sodium hydroxide (NaOH) or ammonium hydroxide (NH_4OH). Experimental conditions are critical and depend on the type of Fe-salt precursors *viz.* chlorides, sulphates, nitrates or perchlorates and also on the ratio of Fe^{2+}/ Fe^{3+} [26,27]. The co-precipitation method can yield Fe-oxide NMs in the range of 5-20 nm diameter. However, optimizations of synthesis parameters like pH, temperature, and ionic force of the medium are vital towards controlling the size and surface properties of the synthesized Fe-ox NMs [26].

In the sol-gel synthesis method, the surface of the Fe-ox NMs are coated with organic molecules, polymers (*e.g.* poly(vinyl alcohol), poly(lactide-co-glycolide), polyethyleneimine, polymethyl-methacrylate and poly(ethylene glycol), biomolecules (*e.g.* gelatin, chitosan and dextrosan) or inorganic molecules such as silica [26,28]. Generally, water is used as the solvent and the metallic precursors are hydrolyzed by either by the addition of acid or a base. Factors like rate of reaction, temperature, nature of precursors and pH are optimized to control the size of iron oxide NMs. For example, nano-maghemite (γ-Fe_2O_3) particles with size ranging from 6 to 15 nm are produced at 400°C [29]. Microemulsion method is primarily used to produce catalytic Fe-oxide NMs. This process yields cubic or spherical particles with characteristic narrow pore size ranging from 4 to 15 nm and high surface area (~315 $m^2\ g^{-1}$) [30,31]. Water-in-oil microemulsion consisting of a cationic or non-ionic surfactant (Triton-X), a co-surfactant (glycols, hexanol, 1-butanol), oil phase (n-octane, cyclohexane) and the aqueous phase is generally used to prepare magnetic iron oxide nanoparticles with controlled size and morphology [32]. The microemulsion is formed through the addition of an aqueous solution with iron salt precursors. Experimental parameters *viz.* temperature, pH, reaction medium, washing cycles, etc. are pivotal in the execution of this process [26,28,30]. The water-in-oil microemulsion methods hold much interest in Fe-ox NM synthesis due to its precise control on size and size distribution of the synthesized nanoparticles (range: 7 - 10 nm) [26,33].

Thermal decomposition of precursors *viz*. ferric acetylacetonate, iron nitroso phenylhydroxylamine or iron pentacarbonyl in octyl ether and oleic or lauric acid followed by oxidation, leads to the formation of Fe-oxide nanoparticles with a size distribution between 4 and 16 nm. The reaction of ferric acetylacetonate in phenyl ether at 538 K in the presence of alcohol, oleic acid and olamines produc Fe_3O_4 nanoparticles that may easily be transformed in γ-Fe_2O_3 by annealing at 523 K and oxygen for 2 h. This preparation method produces monodispersed particles with a narrow size distribution but has a great disadvantage that the resulting particles are always dissolved in non-polar solvents [26,28].

Maghemite (γ-Fe_2O_3) particles are extensively used in pollution control due to their ability to consume secondary pollutants and chemicals from industrial wastewater [26]. The Fe-oxide NMs in the size distribution range of 10-80 nm are also used as solid-gas-liquid phase catalysts in industries for enhanced production of styrene, photocatalytic production of hydrogen and oxygen, catalytic conversion of methane in aromatic compounds, fuel cells and production of biodiesel [26]. Iron oxides are commonly applied as ballistic additives in composite solid propellant formulations in order to accelerate the combustion at the burning surface and so to increase the propellant burn rate and its thrust-time curve. In the field of solid propulsion, Fe-ox NMs like hematite (α-Fe_2O_3) and maghemite (γ-Fe_2O_3) are effectively used as burn catalysts to fuel propellers [26,34]. Moreover, Fe-ox NMs are also applied in several biomedical operations (*e.g.* drug delivery), and nuclear magnetic resonance imaging [35].

3.3 Copper Oxide (CuO) NM

CuO is a higher oxide of Cu naturally found as the mineral tenorite. The compound has a characteristic black color and a melting temperature >1200°C. Generally, it is formed by burning Cu in O_2 available environment as:

$$2Cu+O_2=2CuO$$

In laboratory conditions, CuO NMs are generally prepared through precipitation method [36]. Metallic salts *viz.* $CuCl_2$ and $Cu(NO_3)_2.3H_2O$ dissolved in deionized water are adjusted to pH 14 by adding NaOH till a black precipitate occurs. This precipitate is then washed with deionized water and absolute alcohol until neutral reaction (pH=7) is attained. Later the residue is dried at 80°C and calcined at 500°C for 4 h to yield CuO NMs. Copper (II) oxide is used as a glazing and coloring agent in the ceramic industry and also to sanitize materials for its superb anti-bacterial properties [37]. Cu-oxide NMs in the form of Cu-ammonium hydroxide is utilized used in the production of rayon.

The Copper (II) oxide NMs are effectively used as catalysts in rocket propellants due to its superior properties like uniform propellant combustion rate and low-pressure index. Considering its narrow bandgap (~1.2 eV), Cu-oxide NMs are extensively used as p-type semiconductors. Other important applications of Cu-oxide NMs are seen in the preparation of batteries (both dry and wet cell) and as polishing agent for optical instruments. Additionally, this nanomaterial is extensively utilized during welding operations with copper alloys [38].

3.4 Magnesium Oxide (MgO) NM

Magnesium oxide (MgO) also called magnesia, is a solid white hygroscopic mineral that occurs in nature as 'periclase'. It consists of a lattice of Mg^{2+}ions and O^{2-} ions linked by ionic bonds. The general reaction can be seen as under:

$$MgO + H_2O \leftrightarrow Mg(OH)_2$$

Magnesium hydroxide is produced in the presence of water and this reaction can reversely yield MgO by heating it to separate moisture. Due to its high boiling point (~3600°C), Mg-ox NMs are widely used in the refractory industry. There are few dense engineering ceramics of the structural type made from pure magnesia [39]. Mg-oxide NMs are also used in chemical, construction, environmental and electrical appliance industries.

3.5 Zn-oxide (ZnO) NM

ZnO is known to be a highly functional, and versatile inorganic material in nanotechnology due to its unique properties *viz.* optical, chemical sensing, semiconducting, electric conductivity, and piezoelectric properties [40]. Zn-

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oxide NMs are prepared through different routes but the most followed methods include thermal evaporation of ZnO powders at 1400°C, hydrothermal synthesis, sol-gel technique, simple thermal sublimation, self-combustion, polymerized complex method, vapor-liquid-solid technique, double-jet precipitation, and solution synthesis [40-42]. Interestingly, different production methods yield variable structural configuration of the ZnO-NMs and thus selection of production method varies greatly on the basis of the desired application. Moreover, the optimization of vital parameters *viz*. solvent type, salt precursors, pH, and the temperature is important for a controlled yield of this NM. As mentioned earlier, several successful structural re-arrangements of ZnO-NMs have been synthesized by changing the physic-chemical parameters of the synthesis method. Some of the major configurations of the ZnO nanostructures are nanorods, nanosphere, nanotubes, nanowires, nanoneedles and nanorings [42].

Zn-oxide NMs are very popular as an antibacterial/antimicrobial agent and hence used widely in medicine and cosmetic industries. The “calamine” lotion is prepared out with Zn-oxide powder. ZnO is also utilized in the manufacture of rubber and cigarettes. It is recently used as a preservative of food items and as a coating agent in paint industries. Other important applications of ZnO NMs are in the manufacturing of concrete and ceramics where it is used as an additive.

4. NMs in Soil Environment

Increased demand of NMs has severely escalated their production and as a result, most of these NMs are released into the environment, leading to nano-pollution [43,44]. According to Keller et al. [45], about 9~37% and 63~91% of NMs are directly introduced into the air and landfills respectively as byproducts of industrial activities every year. The major source of NP deposition onto land is currently through the disposal of wastewater treatment (WT) sewage sludge, where NPs that are released from consumer products into wastewaters may partition into sewage sludge during the wastewater treatment process [8,46]. Figure 4 represents a schematic diagram of the different routes of NM introduction to the environment. For instance, the textile industry releases Ag-NMs during washing. According to Benn and Westerhoff [47], the amount of washed Ag-NM is directly related to the agents used for washing as well as the method which is utilized for impregnating the textile with Ag-NM. Similarly, TiO_2-NMs, popularly used in the paint industry is easily leached as effluents into the water bodies [48]. Such industrial effluents eventually end up in a treatment facility and finally sludge is obtained that is loaded with NMs [49]. According to Johnson et al. [50], about 99% of TiO_2-NMs entering the environment are mediated through water treatment sludge.

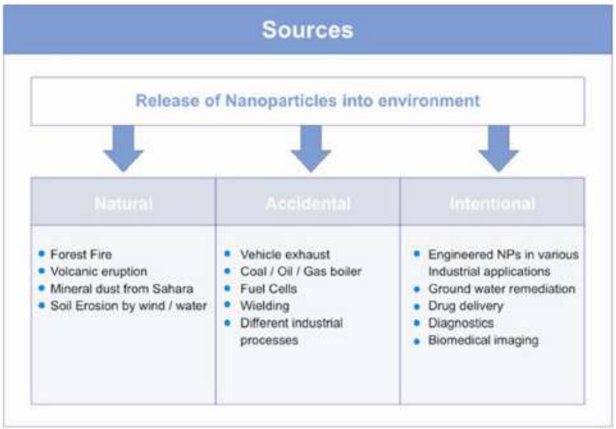

Figure 3. Routes of contribution of engineered NMs (ENM) into the environment

Source: Smita et al. [51] (Permission not required under the open access agreement) (© 2012, BioMed Central, UK)

NMs are introduced in the soil environment mainly through land-disposal of sludge and effluents [46,50,52]. Nanoparticles are unlikely to enter into the soil in their original form, however, because of the organically rich and reactive environments of WTs [53]. In a reduced environment, AgS-NMs are also formed in the sewage sludge after Ag-NMs react with sulfide [54]. Recently, several models are used to predict the fate and final concentration of several NMs into the environment. For instance, according to one model about 1 mg kg^3 and 120 mg kg^3 of Ag and TiO_2-NMs are annually introduced to arable lands when sewage sludge is used as an organic amendment [55]. On the contrary, the production of Ag-NMs should be checked as Ag concentration should be less in all environmental compartments [49,50]. However, TiO_2 is abundantly found in the environment naturally. According to Tourinho et al. [8], about 0.02 to 5.5% TiO_2 is present in 845 top-soils in Europe. Although it is difficult to predict the actual estimates, but TiO_2 and ZnO are the most abundant NMs in the environment matrices owing to their wide applicability [49,50].

5. Behavior of NMs in Soil

Soil is a porous and biologically active natural medium which acts as a sink for moisture, nutrients, and organic matter. The dynamic nature of soil makes it one of the best platforms for understanding the physico-chemical behavior of NMs. Soil comprises of mainly liquid and solid phases and NMs when introduced into soil interact differently with these phases, i.e. NMs either tend to

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aggregate or disperse when introduced into solid/liquid phases. From an ecotoxicological perspective, it is pivotal to understand how specific organisms would react when exposed to NMs in different phases (i.e. soil or soil water). As stated, attributes *viz*. agglomeration/aggregation, dissolution rate, area-charge -surface chemistry of the NMs should be learned to enumerate their eco-toxicity potential. According to Stone et al. [56] information about behavior of NMs in environmental matrices is vital in order to ascertain their stability as well as transport on a spatiotemporal scale.

5.1 Aggregation and Agglomeration of NMs in Soil Matrix

The property of the NMs whereby these bind strongly together by the core is called aggregation. On the other hand, when the bond is formed between the NM surfaces by weak Van der Waals it is termed as agglomeration [8,57]. Generally, agglomeration arises when the kinetic energy of attraction exceeds the repulsive energy [58]. However, when attached by the cores the NMs resemble flocks of particles eventually settled down by gravity [59]. According to Tourinho et al. [8] these particle flocks vary significantly in regard to size and this is mostly dependent on two factors: (1) concentration of the NM in the medium, and (2) size of each NM. Figure 4 presents a schematic view of the major factors that influence agglomeration/aggregation and dissolution processes of NMs.

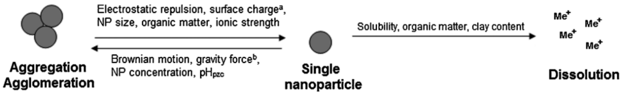

Figure 4. Factors affecting the processes of aggregation/ agglomeration and dissolution of single nanoparticles. ^{a} Considering similar surface charge. ^{b} Acting only on larger particles

Source: Reproduced from Tourinho et al. [8] with permission (©2012, John Wiley and Sons, USA)

Iron oxide NMs readily aggregate with the increase in their concentrations and such aggregations are quite stable[60]. Zn-oxide NMs also tend to form stable aggregates of varied shapes and sizes at higher concentrations [61]. Moerz et al. [62] reported that when Au-NMs were introduced into the hemoglobin solution, the NMs tend to form agglomerates. Moreover, they observed that the extent of agglomeration was influenced by the ratio of Hemoglobin and Au NM concentration in the solution. A pictorial interpretation from their study has been shown in Figure 5.

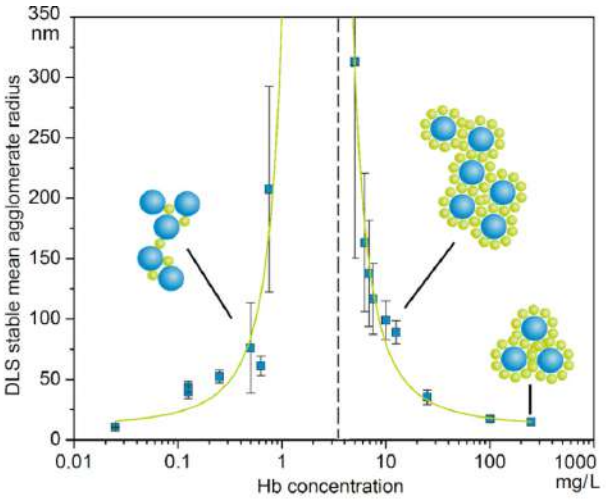

Figure 5. Mean agglomerate radii of stable AuNP clusters from DLS as a function of Hb concentration at a constant AuNP concentration of 16.15 mg/L

Note: Error bars indicate the size distributions calculated from DLS. The green line is a guide to the eye. The Hb concentration theoretically required for monolayer coverage of the AuNP is indicated as a vertical dashed line. Blue spheres represent AuNP, green spheres represent Hb, and both are not to scale.

Source: Moerz et al. [62]. For further reading please visit https://pubs.acs.org/doi/abs/10.1021/acsnano.5b01043 (© 2015, American Chemical Society, USA)

However, the agglomeration/aggregation in NMs varies greatly with the type of NMs. For example, TiO_2-NMs form aggregates of uniform sizes while whereas aggregates of varied sizes are formed when Zn-oxide NMs are introduced in a solution [8,61,63].

5.2 Surface Properties

The surface of NMs is a major attribute that not only dictates its reactivity but also its stability in any medium. Nowadays several materials are used for coating NMs which significantly modify the characteristics and behavior of the NMs in the environment. Hence, studying the time-dependent stability of coated NMs is an important step. Recently, several coated NMs are used as adsorbents for several environmental pollutants [8].

The stability of NMs is duly explained by the DLVO (Derjaguin, Landau, Verwey, and Overbeek) theory. According to this theory the particle-to-particle repulsion as well as attraction phenomena greatly regulate the stability of NMs in various mediums [8]. This theory can also be applied to understand the behavior of NMs in the aqueous medium [64,65]. Although this theory is applicable to most of the NMs, it has been less applied to interpret the behavior of colloidal NMs in soil matrices.

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5.3 Dissolution and Transport

Dissolution of NMs signifies the movement of ions from the core through the electrical double layer into the medium [8,66]. The dissolution of NMs is greatly influenced by the thermodynamic instability of the NMs and also by properties of the medium. Hence, Tourinho et al. [8] advocate the importance of understanding the dissolution patterns of NMs to gauge their toxicity on organisms over time.

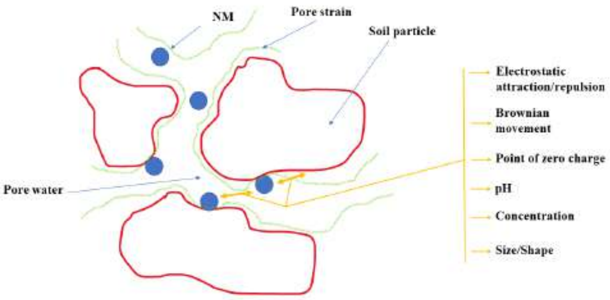

Figure 6. Schematic diagram representing the factors which effect the dissolution/transport of nanomaterials between soil and pore water

Zhang et al. [67] opined that MONMs which are mostly utilized in industries like ceramics, catalysts, coatings, powders, and metal should magnify by 100 folds by 2020. In the UK, several industries like medicine, electronics and sensors, cosmetics, fuel additives, catalysts, paints, etc. utilize huge quantities of metal oxide based NMs [68]. According to Schmid and Riediker [69], the Swiss food and paint industry use MONMs like Al-Ox, Fe-Ox, SiO_2, TiO_2, and ZnO at 1 ton per year per company.

6. Bioaccumulation of Metal based NMs

Mostly, the NMs released/introduced into the environment eventually enter the food chain and get accumulated in the living organisms [6]. In general, plants are used as potential models for studying the toxic behavior of the NMs in terrestrial and aquatic environments. *Brassica juncea* exposed to Mo-NM tend to imbibe the particles culminating into significant inhibition in growth [70]. According to Larue et al. [71], plants can also imbibe NMs through the leaves leading to phyllosphere mediated NM transport. They observed that foliar application of TiO_2 and Ag-NMs in *Lactuca sativa* induced no lethal effects with regular glutathione (GSH) and phytochelatin activity.

Fishes are deemed as potential models for studying the toxicological aspects of NMs in animals. However, there have been numerous studies of NM exposure to nematodes and their fate over time. Recently, biomagnification of Au-NMs was observed in hornworms when they were fed tobacco leaf impregnated with Au-NMs [72]. Their study revealed that the dissolution of Au-NMs mostly varied on the basis of their sizes and less on the zeta-potential. Similarly, TiO_2 NMs form stable aggregates in soil solution and subsequently accumulate in the body of organisms. These NMs bind with lipids and carbohydrates and get biomagnified at each trophic level [8,18]. In nematodes, the negatively charged cuticle attracts NMs and makes dermal route a very convenient way for metal-based NMs to enter [73]. However, oral intake is the most common or major pathway for NMs to enter inside any organisms body via contaminated food/feed [8]. Interestingly, the response of different organisms when exposed to NMs is significantly different from one another. Similarly, different NMs interact differently with various organisms. For instance, earthworms when exposed to ZnO and TiO_2 NMs tend to accumulate higher amount of ZnO than TiO_2, resembling selective uptake [74]. According to Tourinho et al. [8] the niche of soil organisms may also be another key factor which determines the extent of NM exposure. For example, the collembolans will mainly be exposed to NMs dispersed in soil pore water while earthworms will be interacting with both pore water and soil particles, and woodlice to food (decaying leaf material).

Ag-NMs in higher concentrations create oxidative stress in organisms. *Eisenia fetida* specimens when exposed to both nano-Ag and Ag-salt could accumulate higher amount of nano-Ag into their bodies. Shoults-Wilson et al.[75] (94.21 mg kg(-1reported that such accumulation of NMs is facilitated by the dissolution pattern of both nano-Ag and Ag-salt. Similar reports also illustrate higher toxicity of Ag-NMs when compared to $AgNO_3$ in *Caenorhabditis elegans* [76]. In general, Ag-MN enter the body and rupture the reproductive organelles of the worms. Moreover, beyond a concentration limit of 55 ppm, significant dermal abnormalities occurred in the exposed worm [76].

Although less research has been conducted on Fe-oxide NMs these NMs bear significant potential in biomedical industry [77]. According to Gonzalez-Moragas et al. [78] citrate-coated Fe-oxide NMs induce lethal effects to soil nematodes like *Caenorhabditis elegans.* it was observed that at higher concentration ($500\mu g\ ml^{-1}$) Fe-oxide NMs trigger oxidative stress evidenced through upregulation of genes *viz. sod1, sod 2chc-1, dyn-1, eps-8, act-5*, and *elt-2*. A higher expression of metal-chelating proteins (metallothioneins) is also prominent when these

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nematodes are exposed to higher concentration of Fe-oxide NM. Moreover, Fe-NMs tend to accumulate in the body of *Caenorhabditis elegans* when exposed for longer time periods and this led to significant disruption in locomotory power of the organism [79].

Cu-oxide NMs applied in soil (at the rate of 65 mg kg^{-1}) induced non-lethal effects on *Eisenia fetida* [80]. Gomes et al. [81] observed that Cu-NM at 1g L^{-1} induced no toxic effects to *Enchytraeus albidus.* However, higher concentration (>100 ppm) of Cu-NMs accelerated deterioration of biomolecules (*i.e*. carbohydrate, lipid, and protein) in *E. albidus* after 6 weeks of exposure. Gomes et al. [81] proposed that Cu-induced toxicity was mainly driven by the undissolved Cu-NMs. They even traced damage at the molecular level (gene expression) suggesting the deleterious impact of Cu-NMs on exposed organisms. Generally, oxidation of Cu-NMs yields Cu-ions which might be the cause of such toxic effects [8].

ZnO is a highly investigated NM regarding ecotoxicity enumerations in living organisms [8]. Several workers have observed genetic and physiological changes (mainly stress enzymes *viz.* catalase, superoxide dismutase, peroxidase) in *Eisenia fetida* exposed to ZnO NMs [8,74]. Hooper et al. [82] compared the toxicity of ZnO NM and $ZnCl_2$ on growth and proliferation of *E. veneta*. They reported that soil spiked with ZnO NM reduced the reproduction of *E. veneta* by 30% but did not hamper their immunity. It was confirmed through SEM and EDX that ZnO-NMs got accumulated in the body tissue through an internal mechanism [82]. A similar report by Canas et al. [83] also elaborated on the deleterious effects of ZnO NMs to *E. fetida* at higher concentrations, especially in the form of oxidative stress. In case of the isopod *Porcellio scaber,* nano-Zn accumulation was primary routed through the dissolution of ZnO-NMs rather than direct accumulation of the particles [61,84]. Contrarily, Manzo et al. [84] extended information on the accumulation of dissolved-Zn species *in lieu* of particulate-Zn in the body of *Folsomia candida* exposed to ZnO-NMs.

7. Summary

Although the use nanomaterials are going on for quite a 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 of how such discharged nanomaterials influences the soil physicochemical properties as well as the life forms dwelling in the land ecosystems. Hence, it is become imperative to carry out in-depth research on how nano-materials interact with the biological factors so that efficient and non-lethal nano-materials could be synthesized.

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ARTICLE

A Unit Study of Externality of Shrimp Farming on Provisioning Services (Paddy Farming)

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Externality

ABSTRACT

Externality; the term can define as a positive or negative impact from either production or consumption of goods or services. Services provided by particular location have very specific dependency on spatial characteristics of that region. A region's distinct characteristics make it ecologically unique from other such regions. Ecosystem services are offered by these regions thus differ according to these unique ecological features. In this particular study, artificially imposed expansion of coastal shrimp farming towards the inland and its impact over paddy cultivation have been addressed. Optimization of the extent of this manipulative coastal expansion has been supported by little modification of a previously described model. Here the investment prediction for both shrimp and paddy farming has been investigated by calculating net present value (NPV). Shrimp farming has very specific externality on local ecosystem services. In this particular case, some contradictory results are presented and with respect to positive or negative externality; but the externalities are strong. NPV results indicate that there is no long-term profitability in case of shrimp farming. Hence, an overall externality of shrimp farming has been described in context of this study.

1. Introduction

Externalities (also called "spill-over effects") arise when one valuable objective function, like profits from any investment or personal happiness of any person, is directly or indirectly dependent on the unintentional or accidental "by-product" of other's activity [1, 2]. Therefore, whenever any investment (may be short-term or long-term) or any infrastructures are established, the investors always try to minimise externality. Externality is either positive or negative. For internalising the externality, investors or government or institute take extenuation steps. When environmental cost, caused by any industries or activities is not included in policies then no internalisation happened [3]. For dealing with the problem where environment is involved, recovery cost of negative externalities on environment incorporated in decision making and "compromise business community in a long term" [4].

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From the point of view of externality; shrimp farming industries are most controversial one. Huge profit within short period of time and high demand in national and international market is the main driving force behind the ever-expanding shrimp farming industry. Specially, brackish water shrimp culture is a major boost up for coastal economic prosperity. The expansion of shrimp farming has risen as a major trade-off product since 1975. Before that only 2% of the world market was occupied by this major 'blue revolution' agent (shrimp) [5]. In coastal India, different states viz. Gujrat, Andhra Pradesh, West Bengal etc. are emerging their economy by shrimp farming (especially brackish water shrimp farming). The presence of suitable ecological and environmental supports acts as main driving force for this emerging shrimp farming in these states. Though a huge manipulation, in terms of maintenance is needed to succeed in shrimp culture. This maintenance differs according to culture species and culture processes.

In this particular study, the study area is at Paschim Shitalpur village, at Nandakumar subdivision of Purba Medinipur district, West Bengal, India. Major trend of Purba Medinipur district is to convert paddy cultivation land into low depth saline water bodies. As per present statistics there are more than 25000 shrimp farms, most of these farms did not measure any externality. Here, externality comes mainly in negative measures.

In order to investigate the impact of outward expansion of coastal boundary by canalizing the brackish or salt water directly from source to the inward fertile land for shrimp culture on paddy farming; some sort of key objectives have been reached both in qualitative and quantitative aspects. Inward extension of salt water has a huge impact on spatial variabilities. The variation range which occurs in between coastal and non-coastal region is being diminished day by day by this kind of activities. These activities may lead to the alteration of directly or indirectly ecosystem services which are extensive result of these kinds of variabilities. Here, the ecosystem services and optimum expansion range of coastal region are considered. This consideration is very relevant for conservationist for making policy to conserve coastal region as well as mitigate the confliction between coastal expansion and fertile inland conservation. The Net Present Value (NPV) has been calculated and compared by categorising the paddy farming system in different groups with each other's groups and with shrimp farm. From investors perspective in both such choices (paddy farming and shrimp farming) the comparison of NPV value is effective for the projection in long run. Externality in terms of qualitative impacts assessment for intensive or semi-intensive shrimp culture, at the study location, on ecosystem services; act as holistic conclusion of this study.

2. Materials and Methods

2.1 Study Area

The entire study has been conducted at shrimp farming region of Shitalpur Paschim village, Nandakumar block, Purba Medinipur, West Bengal. Shrimp farms are located along a canal which is canalized from River Haldi at a point of 22°08'18.72" N and 88°53'24.35" E and routed in a northward direction for 6.5 km inland of Shitalpur Paschim (Figure 1). The canal is on 25 km upstream or inland from the mouth of River Haldi, where it meets and River Hooghly which is finally draining into Bay of Bengal. Data from paddy farms were collected from adjacent paddy farms of this particular shrimp farm. The location map (Figure 1) is prepared using ArcMap by digitisation of scanned map and data point collected during field survey by author.

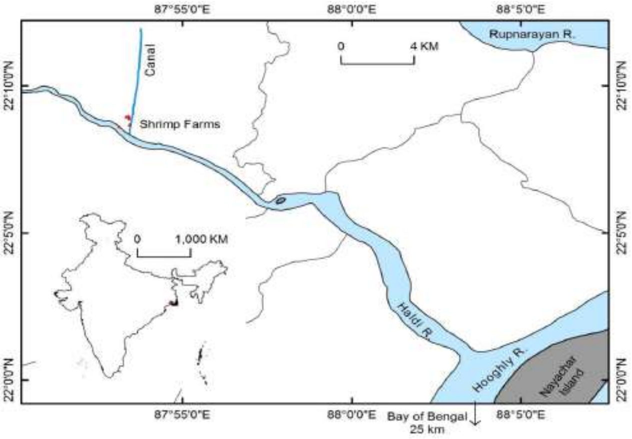

Figure 1. Location Map of Shrimp Farming and Adjacent Paddy Farms (Source: Prepared by ArcMap)

2.2 Data Collection

For collecting data, a primary survey was conducted with the help of interactive face to face questionnaire. Costs and benefits data were collected from a shrimp farm from their previous record books from the year of 2009 to 2018. Paddy farms which are situated beside the particular shrimp farms are considered for the study of externality of brackish water shrimp farming. Lots of other paddy farms have been converted into brackish water low depth ponds from paddy after leasing for shrimp farming. Production cost, benefits and other data (total production, market price, amount of production utilised for their own needs

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etc.) were collected from paddy farmers who are still cultivating at Shitalpur Paschim beside this shrimp farm. Here, these paddy farms are categorised according to their distance from the shrimp farms as follows:

(1) Proximal farm: Near to shrimp farm - within 100 meters and

(2) Distal farm: Far from shrimp farm - more than 100 meters.

2.3 Analysis

First of all, it has been tried to address the spatial pattern of the farm lands and their relationship with ecosystem services. This relationship has been described using a simple mathematical model described by Barbier (2012) with little modification in perspective of this study. By this model, the optimum width of coastal expansion can be measured theoretically, along with the difference of ecosystem services between coastal and inner land (non-coastal area) with respect to spatial variabilities.

By the production cost and benefits data from shrimp farm, Net Present Value (NPV) has been calculated at different discount rates. NPV is an economic methods dealing with discounting of future cash flows [6]. Net present value is the yield from goods or services of time series by subtracting the cost by discount rate. Discount rate is very vital and specific for each decision makers for projection of particular investment. Discount rate is rate of return at which the investor wants to derive the cost of money. Initially, the paddy farms were divided according to distance from shrimp farm, then the NPV calculation for paddy farms, average cost of production, average benefit of production have been estimated by calculating arithmetically according to year wise MSP (Minimum Support Price) of government.

The NPV of shrimp farm and both categories of paddy farms were detected to measure and compare their long lasting approach towards economic profitability [7]. NPV defines the net contribution to the economy of a particular investment. NPV is measured by the sum of year wise net benefit in term of the present value [8]. The formula of NPV is as follow:

$$NPV = \left[(B_0 - C_0) + \frac{(B_1 - C_1)}{1+r} + \frac{(B_2 - C_2)}{(1+r)^2} + \ldots\ldots + \frac{(B_n - C_n)}{(1+r)^n}\right] > or < 0 \quad (1)$$

Where, *B* is the category specific benefits over the year; *C* is the category specific cost over the year and *r* is the discount rate. For determine the social, environmental cost and benefit; discounting the events for the 'distant future' is very important for economic or eco-economic analysis. For this research with the concern with externality caused by shrimp farming has been tried to focus on discounting the payoffs of distant future. This is tricky task to standardise the discounting for the distant future with considering all right and wrong intuitionism [9]. The value of NPV can be greater than or less than 0. The greater than and less than value signifies investment prediction.

3. Observations & Discussions

3.1 Spatial Variability and Ecosystem Services

Derived gains of human society; from ecosystem functions, features and from cumulative interactions of these are known as Ecosystem Services [10] All the dimensions of human society from economic to political, from agricultural to cultural, from religious to developmental are directly or indirectly dependent or interconnected with various ecosystem services.

The characteristic features of an ecosystem are defined by different components, their properties, their assemblage and functioning. If all those ecological and environmental specificity determining factors are compared between different spatial locations, then spatial variability arises. From this context, it can be said that spatial variability depends of different physical properties (e.g. thermal conditions, humidity, soil texture, soil chemistry etc.), biological conditions (e.g. presence of different flora and fauna, microbes, planktons etc.), availability of different nutrients (e.g. litter deposition, nitrogen contents, and mineralization etc.) and others (e.g. weather history, anthropogenic activities etc.). Therefore, the distinctive services provided by any ecosystem differ from other ecosystems as variabilities exists in between all ecosystems from spatial to others determining sectors for example the brackish water shrimp farming flourishes in coastal parts of Purba Medinipur district of West Bengal due to its spatial location (presence of Bay of Bengal and suitable environmental conditions) but it cannot flourish at the inner most region of the same district (due to further distance from the Bay of Bengal and other spatial conditions which are not suitable for brackish water shrimp farming).

From the above-mentioned consequences of spatial variabilities it is clear that the different ecosystem services of different spatial location are intermingled outcome with spatial variabilities. In these aspects, the ecosystem services depend on spatial variability. Even spatial variability differs according to the temporal variations, i.e., the variations of ecosystem services are equally dependent on both spatial as well as temporal variabilities. The services provided by a particular ecosystem were previously considered as followed the linear pattern. Now it has been cleared that ecosystem services follow nonlinear pattern with relation to spatial and temporal

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variabilities [11]. In some consecutive effects, loss of ecosystem services and gradual shrinking of habitats create high risk of collapse on ecosystem.

Amendment in the Indian coastal Aquaculture Authority Act 2005, which stated that "area of land within a distance of two kilometres from the high tide line of seas, rivers, creeks and backwaters," would be classified as a coastal area. But the problem is that the saline water is canalized far beyond the coastal boundary to the inland fertile non-coastal regions at study area, Shitalpur. The canal beside the particular shrimp farm has been shown in Figure 1.

Therefore, it is very pertinent to understand up to which distance the coastal expansion can be optimum. The optimum level can be set by considering persistency of ecosystem services. Considering ecosystem services (*ES*) is the function of spatial variability (S_v).

$$ES=fS_v \qquad (2)$$

From the proposed model [12], the idea of optimization of this expansion can be estimated. Suppose, if any social planner tries to preserve any landscape area which is denoted as (a). The expansion of boundary of the landscape toward sea defined as 0 and toward the main land as A. Considering maximum ecosystem services toward sea, social planner can estimate the optimal width of coastal landscape (W_a).

$$W(a) = \int_0^a (v-m)S(i)di + R(A-a) \qquad (3)$$

Where, $(v\text{-}m)S(i)$ is net benefit [v = value of the ecosystem service $S(i)$, m = cost of maintaining the ecosystem service $S(i)$] of ecosystem service $S(i)$. $R(A\text{-}a)$ is opportunity cost of remaining location.

The Environmental and Ecological uniqueness of Purba Medinipur, which is exclusively suitable for shrimp culture, is majorly defined by convergence of freshwater rivers plains with Bay of Bengal. The presence of Bay of Bengal makes this area distinct from others region and the favourable condition for brackish water shrimp culture is also an ecosystem-services out-come of this region. Though ever-expansion growth of shrimp farming and manipulative semi intensive culture method are major detrimental agents of natural services, hence uncertainty exists in future of shrimp culture and drastic spatial variability with favourable ecosystem services out-come.

3.2 Comparison of NPV of Different Types of Investment

NPV is one of the major indicators for investment. It considers relativity of performances of investors. When the time horizon is fixed, depending on the discount rate a specific magnitude of NPV can be reached. What will be the future value of the money invested today? Or what amount of money should be invested to get certain future value? To answer this question, an investor should always measure present value. Present value can be estimated by discounting the future amount by certain interest rate. This interest rate is called discount rate. To set a perfect discount rate is crucial for NPV calculation.

Here, for comparison between the two categories of paddy farming systems NPV of both farms for equal time periods have been calculated. The basic similarity between two farms is with increasing farming shifting from paddy to shrimp industries; still they are doing paddy farming practice. The distance from shrimp farm is main dissimilarity among these.

In paddy farming, the maximum discount considered is 7%, as the farmers get government's loan with some other suitable subsidies at 7% interest rate. Here, to understand the reliability of nature of NPV, the entire calculations were carried out from minimum to maximum discount rate with some regular interval (at discount rate 1%, 3%, 5%, 7%) and the comparative result presented in Table no. 1. The time period has been considered 10 year unto 2018 and cost and benefits were standardised in equal margin of land amount and market price.

Table 1. NPV of two categories of farming system at different discount rate

Categories of paddy farms system	**NPV at different discount rate in Indian currency (Rupees ₹)**			
	1%	3%	5%	7%
Proximal farm system	130827.812	125198.633	120226.898	115812.345
Distal farm system	-27298.546	-21610.287	-16812.987	-12754.67

NPV has been calculated for the particular shrimp farm for same time horizon and equally standardised to maintain equality with paddy farms but here the maximum discount rate is 30%. As the shrimp farmers take loan from unauthorised sector (not have government authorisation). Here, the NPV of shrimp farming system has been estimated with maximum 30% rate and minimum 7% rate (the minimum rate is the maximum rate of paddy culture industry).

Table 2. NPV of shrimp farming system

Shrimp farming system	**NPV at different discount rate in Indian currency (Rupees ₹)**	
	7%	30%
	-17827189.43	-8441325.967

From above mentioned results of NVP, some of contradictory conclusion may be arisen. First of all, in case of shrimp farming system the NPV shows it is less

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than zero in both maximum and minimum discount rate. Hence, from this result it can be very clearly predicted that there is no such future profitability. In case of paddy farming system, the proximally located farm system has positive NPV values at all of the different discount rates but the distally located farm system has completely opposite result. The distally located farm has negative NPV at all of the different discount rates.

It is wide spread that intensive or semi intensive culture of brackish water shrimp culture always have some negative externality towards the native provisioning services [13]. Here, this unit study come up with completely contradictory results. With consideration of previously studied results, it was very natural prediction that due to the some detrimental effects of shrimp culture like saline water intrusion, unwanted exposure of certain chemicals used in shrimp farms etc., the NPV of proximal farming system should be negative and lower than the distal one. Here, the opposite and the contrast between the NPV results of these two farming systems indicate some probable possibilities behind this occurring:

(1) Might be some sort of positive externality of shrimp farming present on proximal farming. Or

(2) The proximal farming land or culture land of shrimp farming are itself much more fertile than the other parts but the lands are converted into low depth saline water bodies.

In support of first possibilities, it can be assumed that nutrients from shrimp farms which intrudes in adjacent paddy cultivation land along with water. This nutrients which are deposited, might have some enhancing effects on paddy production and which is yet to be explained by ecologically.

The second assumption has great chance of possibilities and can be easily supported by previous works which had been carried out at different parts of world. In this respect most suitable explanation was come by the following statement of J H Primavera (1997):

"When intensive farming is practised, the life span of ponds does not exceed 5-10 years because of attendant problems of self-pollution and disease. In some cases, entrepreneurs have moved on to other areas in a pattern called 'rape-and-run' and the sterile lands are no longer available for agriculture or aquaculture."

When any one compare different services values including all trading and non-trading values with before and after shrimp culture period of a particular ecosystem, then we can make some conclusions.

3.3 Externality and Shrimp Culture

The perception of externalities deals with analysis of connectivity of economic welfare. In existence of externalities costs and benefits of each individual may vary in respect of consequences of true social cost action in them. According to Pigou, externalities seem as one of the principal reasons of deviation among "private net product" and "social net product" [14]. In case of shrimp farming the holistic externality concept lies with assembling both social and ecological interfaces with economic welfare. Negative externality of shrimp culture had been repeatedly reported from different leading countries of aquaculture since after blue revolution occurred in the World. In south-east Asian countries (Sri Lanka, Bangladesh, India etc.) have been reported salinization and hardening of shallow well for domestic and farm land water supplies [13,15].

The trend of shrimp farming has negative externalities in terms of enrichment of nutrients in marine water and lead marine eutrophication, long term exposure of chemicals and toxic products cause changes in abundance of local non- target species, chance of creation of some invasiveness by introduction of exotic species, creation of extreme threats on mangrove ecosystem etc. The wide spread expansion of coastal shrimp culture is altering the multiservice functions provided by mangrove into fragmented ponds which has given some artificially driven production services and at the same time degrade the ecosystem [16].

The sustainability of shrimp culture as well as sustainable aquaculture refers the both ecological and economic sustainability. Both these sustainability scenarios are centred to the long run goal. In case of the semi-intensive or intensive method of shrimp farming the farmers adopted this practice because its high income is short period. For the illusion of high-income farmers neglected their farsightedness to measure its longevity. They are courageous to take the risk of huge investment and huge loss.

In this particular study, it is very difficult to draw any strong conclusion of positive or negative externality on the basis of assessment of the contradictory NPV values of both farming systems. To make such conclusion different ecological and environmental parameter and their interactive nature with all socio-economic behaviour must be investigated. Concept of sustainability with incorporation of shrimp culture and its externality must be studied with some implications of strong or weak sustainability concept.

4. Conclusion

This is a unit representation of entire ever-growing shrimp farming of Purba Medinipur. It stretches a scenario

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of condition of shrimp farming and paddy culture in future. In further extension of this study to understand the externality of shrimp farming NPV data of paddy farms adjacent to the shrimp farming region would be compared with the NPV of paddy farming region, situated completely out of the externality of shrimp farms. The value added by the altered practice from paddy to brackish water shrimp farming would be compared in next face of this work with the value added by the previous paddy culture practice. Ultimately the social cost would be estimated and incorporated in determination of actual cost and benefits of shrimp farming. In terms of social cost the ecological and environmental damages, ecosystem services alterations all would be integrated. Therefore, the holistic measurement actual cost and actual benefits of shrimp farming would be assessed. In the mean times; the externality of shrimp farming weather it causes negative externality or positive externality; would be cleared by this incorporation of social and ecological and environmental costs. Different ecosystem services valuation methods to measure both altered and actual service value is pertinent steps of this aspect.

Finally, this study and its extension would be the basic footsteps for implementation of policy which addresses the all externality of shrimp farming in this location to mitigate the problems.

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ARTICLE

Influence of Briquette Fertilizer of Levels of Nitrogen and Silica on Paddy

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ABSTRACT

Nitrogen is the major nutrient plays a key role in the production of rice crop and the lateritic soils are low in available nitrogen content. Accordingly, the field study was carried out in lateritic soils of Konkan (*Coastal*) region during year (2017 - 2018) to find out the effect of different levels of nitrogen along with silica levels on growth and yield of rice. The major aspect behind taking this kind of research was to see the interaction between the applications of varying levels of nitrogen with the different levels of silica. An experiment was carried out with FRBD design. The nitrogen was given by the Konkan Annapurna Briquettes (KAB) which is the compressed tablet type mixture of the fertilizer which is having major nutrients N, P and K. Silica was applied solely through potassium silicate with the paper packets. There might have been positive response with growth and silica levels also with the rate of application of nitrogen with the increased levels of silica. It was observed that the application of 80 per cent RDN through Konkan Annapurna Briquettes (KAB) with Silica @ 100 kg ha-1 which was applied between four hills was found promising in enhancing the growth and yield of Ratnagiri-24 Cv. in lateritic during *Kharif* season. It could be concluded that, the application of nutrients in the form of Konkan Annapurna Briquettes along with the application of silica can reduce the recommended dose of fertilizer to the extent of 20 per cent during *Kharif* season in Konkan region.

1. Introduction

Rice (*Oryza sativa* L.) belonging to family *Poaceae,* one of the most important staple food grain crop of the world. Rice is also an important cereal food crop of Maharashtra state, which contributes 3.6 per cent of area and 2.8 per cent of production of rice at national level. In Konkan region, rice occupies an area of 3.79 lakh hectares with production 9.94 lakh tonnes and productivity of 2.61 tonnes ha^{-1} [2]. Nitrogen fertilization plays a great role in increasing rice production. Nitrogen is one of the most mobile plant nutrients in the soil as well as in plant also. Nitrogen impact green colour to plant encourages vegetative growth. Nitrogen is essential constituent of protein. It is also constituent of protoplasm of chlorophyll and co-enzyme. It plays important role in synthesis of Auxin. The total nitrogen uptake and the nitrogen use efficiency by hybrid rice is also higher [11,34].

In high rainfall regions like Konkan, there are heavy

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losses of fertilizer N applied by broadcast method through various mechanisms *i.e.* runoff, volatilization, leaching and denitrification resulting into poor nutrient recovery [33].

Earlier studies carried out in this university indicated that the placement of N and N+P through paper packets were used [3], urea placement behind plough was modified after the application through packets [30,32] and deep placement of urea super granules (USG) and UB-DAP [25] seemed very promising in order to improve the NUE and nutrient recovery in transplanted rice. Therefore, it would be better if all the three major plant nutrients are used in the briquette form. Presently there is the myth that the farmers are applying high dose of Nitrogen, beyond recommended doses ranging from 160 to 220 kg ha^{-1} but in fact the farmers are using less fertilizers than the recommendations even. Yes obviously it is true in the coastal region of konkan farmers are using the nitrogenous fertilizers mostly at high doses, hence the N use efficiency may be low besides leading to other ill effects of lodging and susceptibility to pests and disease. These effects could be minimised by use of silicon, so application of silicon is found to be improved N use efficiency besides reporting resistance to pest and diseases (Rajamani, 2012).

Silicon is added to plants as a fertilizer, which can be in liquid or solid state usually applied at the time of planting, but it can be applied at any time during the growing season. Here in this university one ongoing experiment is being taken by the scientist where it was noticed that the foliar application of silica increases the yield of rice with higher concentration in single spray at tillering stage. Silicon fertilizers in agriculture are still not widespread and they are considered as a modern farm technology, side by side with microbiological fertilizers. Since it's a natural element, silicon based fertilizers can use all farmers, whether they practice integration, conventional or organic farming. Information on the importance of Si in Indian rice farming system is limited [21]. There is need to identify silicon deficient soils, for determining desired rates of silicon fertilization and for assessing various silicon fertilizer sources. Rice and sugarcane are Silicon (Si) accumulator plants. No other crop requires as high Si as required by rice and sugarcane.

Si plays a noteworthy role in conveying both biotic and abiotic stress resistance and boosts productivity. Si is also an element which is worked as potassium that does not damage plants upon its excessive accumulation. High accumulation of Si in rice has been established to be necessary for healthy development and great steady production. For this reason, Si has been recognized as an "agronomically essential element" in Japan and silicate fertilizers have been applied to paddy soils [12]. Rice is a known silicon accumulator [29] and the plant is benefited from Si nutrition [27]. Consequently there is a definite need to think through it for considering as an essential minor element to increase sustained rice productivity [28].

Several studies suggest that Si enhances disease resistance in plants, imparts turgidity to the cell walls and has a putative role in mitigating the metal toxicities [4,5]. It is also advised that Si plays a vital role in checking the lodging in the cereal crops, a matter of great importance in terms of agricultural productivity.

Seven international conferences on SILICON IN AGRICULTURE were organised at many parts of the world since 1999. As such, many of the plant scientists from India are aware of the importance of silicon in agriculture especially as it relates to plant health and soil productivity. In India, though research on silicon has been initiated earlier, the necessity for silicon fertilization to the rice crop has not been widely evaluated as in other countries.

2. Material and Methods

A field experiment was conducted during *kharif* season 2017 at Experimental Farm, Department of Soil Science and Agril. Chemistry, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri which is situated in subtropical region at 17^{0}19' North and 73^{0}1' East longitude having altitude of 250 meters above mean sea level. The climate is subtropical, warm and humid which is favourable for rice crop during *kharif* season. The average annual precipitation of Dapoli is nearly 3150 mm distributed from the beginning of June to October in about 95 to 110 days. The soil of experimental site was Sandy clay loam in texture with pH 5.21, EC 0.17 dS m^{-1}, Organic Carbon 16.1 g kg^{-1}, available nitrogen 406.98 kg ha^{-1}, phosphorus 20.18 kg ha^{-1}, potassium 381.02 kg ha^{-1} and Silica (acetic acid extractable) 310.12 kg ha^{-1}.

The experiment was laid out in factorial randomized block design comprising of sixteen treatment combinations with three replications. The four levels of Nitrogen through KAB and four levels of Silica through potassium silicate were used for this experiment. Rice (*Oryza sativa* L.) var. Ratnagiri-24 was taken as a test crop during *kharif* season 2017 with a spacing 15 x 20 cm^{2}. In order to study the effect of various treatments on the growth parameters, yield contributing characters and yield were recorded at 30 DAT, 60 DAT and at harvest. The details of the treatments are given in the table 1.

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Table 1. The details of the treatments

Sr. No.	Treatment Combination	Description of treatment Combination
1	N0Si0	Absolute control
2	N0Si1	Silica 50 kg ha-1 through potassium silicate
3	N0Si2	Silica 75 kg ha-1 through potassium silicate
4	N0Si3	Silica 100 kg ha-1 through potassium silicate
5	N1Si0	40% RDN through KAB
6	N1Si1	40% RDN through KAB + Silica 50 kg ha-1 through potassium silicate
7	N1Si2	40% RDN through KAB + Silica 75 kg ha-1 through potassium silicate
8	N1Si3	40% RDN through KAB + Silica 100 kg ha-1 through potassium silicate
9	N2Si0	60% RDN through KAB
10	N2Si1	60% RDN through KAB + Silica 50 kg ha-1 through potassium silicate
11	N2Si2	60% RDN through KAB + Silica 75 kg ha-1 through potassium silicate
12	N2Si3	60% RDN through KAB + Silica 100 kg ha-1 through potassium silicate
13	N3Si0	80% RDN through KAB
14	N3Si1	80% RDN through KAB + Silica 50 kg ha-1 through potassium silicate
15	N3Si2	80% RDN through KAB + Silica 75 kg ha-1 through potassium silicate
16	N3Si3	80% RDN through KAB + Silica 100 kg ha-1 through potassium silicate

Note:
(RDN- Recommended dose of Nitrogen; KAB- Konkan Annapurna Briquette)

The Konkan Annapurna briquettes were prepared as per the ratio of fertilizers combination used with the help of "Kranti Briquetter". The 1:1.5 (Godavari to Urea) ratios was used for preparation of briquettes. The details like average length, breadth, shape and Ratio of fertilizers used for briquette preparation are given in table 2.

Table 2. Compositon and shape of briquette

Sr. No	Briquette (Proportion)	Ratio	Avg. Length (cm)	Avg. Breadth (cm)	Avg. Weight (g)	Shape
1	Godavari (14:35:14) : Urea	1:1.5	3.10	2.13	2.71	Oval Flat

2.1 Briquettes and Silica Application

Treatment wise Konkan Annapurna Briquettes (KAB) and potassium silicate was placed manually at about 7 to 10 cm @ one briquette for KAB and one paper packet of potassium silicate for every four hills of rice at the spacing of 15 x 20 cm^2.

2.2 Statistical Analysis

The data have been analyzed with appropriate method of statistical analysis [18]. Interpretation of result was based on 'F' test. The comparison among means was made by calculating critical difference (CD) at 5 per cent level of significance.

Table 3. Nutrient content in various fertilizers and briquettes

Sr. No	Fertilizer/Briquette	Nutrient content (%)			
		N	P_2O_5	K_2O	Si
1	Konkan Annapurna briquette	34	14	6	-
2	Potassium silicate	-	-	32	52

Table 4. Total nutrient added in each treatment

Sr. No	Treatment Combination	N (g Plot-1)	P2O5 (g Plot-1)	K2O (g Plot-1)	Si (g Plot-1)
1	N0Si0	-	-	-	-
2	N0Si1	-	-	187.5	115.38
3	N0Si2	-	-	281.25	173.08
4	N0Si3	-	-	375.00	230.76
5	N1Si0	141.17	19.75	8.47	-
6	N1Si1	141.17	19.75	195.97	115.38
7	N1Si2	141.17	19.75	289.72	173.08
8	N1Si3	141.17	19.75	383.47	230.76
9	N2Si0	211.76	29.54	12.66	-
10	N2Si1	211.76	29.54	200.16	115.38
11	N2Si2	211.76	29.54	293.91	173.08
12	N2Si3	211.76	29.54	387.66	230.76
13	N3Si0	282.35	39.48	16.92	-
14	N3Si1	282.35	39.48	204.42	115.38
15	N3Si2	282.35	39.48	298.17	173.08
16	N3Si3	282.35	39.48	391.92	230.76

3. Result

3.1 Effect of different Levels of Nitrogen and Silica on Growth Parameter of Rice

The data presented in table 5, showed the statistical variations in the plant height at various stages of the crop with the application of varying levels of nitrogen as well as silicon. The application of different levels of nitrogen showed significant result with respect at 30 DAT. The maximum plant height of rice (71.32 cm) was recorded in the N_3 treatment in which 80 per cent RDN through KAB was applied. Similarly the application of different levels of silica showed significant results with respect to plant height of rice. The maximum plant height of rice (71.23 cm) was recorded in the Si_3 treatment in which 100 kg Si ha^{-1} was applied. The height of rice was increased gradually from 48.03 to 76.00 cm in all treatment combinations.

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Table 5. Effect of different levels of nitrogen and silica on plant height at 30 DAT, 60 DAT and at harvest

Treatment Level	**Plant height (cm)**		
	30 DAT	60 DAT	At Harvest
N0	59.98	100.41	101.83
N1	66.77	110.97	111.27
N2	70.02	117.49	118.38
N3	71.32	118.81	120.03
Mean	67.02	112.17	112.88
SE	1.13	1.19	1.38
CD	3.25	3.44	3.99
Si0	62.24	106.25	107.42
Si1	65.33	110.32	111.95
Si2	69.27	114.99	115.22
Si3	71.23	116.12	116.93
Mean	67.02	112.17	112.88
SE	1.13	1.19	1.38
CD	3.25	3.44	3.99

The interaction effect was found statistically not significant. In the interaction effect, the maximum height of plant (76.00 cm) was recorded N_3Si_3 treatment combination in which 80 per cent RDN through KAB with potassium silicate @ 100 kg ha^{-1} was applied. At 60 DAT and harvest, similar results were observed. The application of different treatment combinations did not reach the level of significance with respect to height of plant. The maximum plant height at 60 DAT (118.81 cm) and at harvest (120.03 cm) was recorded in the N_3 treatment in which 80 per cent RDN through KAB was applied.

The application of different levels of silica showed significant results irrespective of plant height of rice. The maximum plant height was recorded at 60 DAT and harvesting stage (116.12 and 116.93 cm) in the Si_3 treatment where Si was applied @ 100 kg ha^{-1}.

3.2 Number of Tillers

The data depicted in table 6, showed the variations in tillering of the crop at various stages with the application of varying levels of nitrogen as well as silicon. The application of different levels of nitrogen showed significant results irrespective of number of tillers. The highest number of tillers was recorded at 30 DAT, 60 DAT and harvest (8.02, 10.78 and 9.82) in the N_3 treatment in where 80 per cent RDN through KAB was applied. The maximum number of tillers at 30 DAT (7.95), 60 DAT (10.70) and at harvest (9.52) was recorded in the Si_3 treatment where Si was applied @ 100 kg ha^{-1}. It was observed that the application of different treatment combination did not reach the level of significance at all growth stages of rice with respect to number of tillers. The number of tillers decreased at harvest stage than the 60 DAT because at 60 DAT measured tillers what is observed *i.e.* not complete panicle stage but at the time of harvest measured the effective tillers per hill *i.e.* panicle are developed so these decreasing the number of tillers at harvest than 60 DAT.

Table 6. Effect of different levels of nitrogen and silica on Number of tillers 30 DAT, 60 DAT and at harvest

Treatment Level	No of tillers		
	30 DAT	60 DAT	At Harvest
N0	5.62	7.70	6.85
N1	7.77	9.80	8.67
N2	7.80	10.17	9.45
N3	8.02	10.78	9.82
Mean	7.30	9.61	8.70
SE	0.27	0.19	0.23
CD	0.77	0.56	0.66
Si0	6.65	8.88	8.02
Si1	7.12	8.98	8.22
Si2	7.48	9.89	9.03
Si3	7.95	10.70	9.52
Mean	7.30	9.61	8.70
SE	0.27	0.19	0.23
CD	0.77	0.56	0.66

3.3 Effect of Different Levels of Nitrogen and Silica on Yield of Rice

Application of different levels of nitrogen significantly influenced the grain and straw yield of rice has been showed in the table 7. The significantly highest grain yield (35.99 kg ha^{-1}) and straw yield (48.24 kg ha^{-1}) were recorded with application of 80 per cent RDN through KAB. Application of potassium silicate @ 100 kg ha^{-1} produced the highest grain yield (31.21 kg ha^{-1}) over the different levels of silica but straw yield did not reach the level of significance. The application of different treatment combinations did not reach the level of significance with respect to grain and straw yield. There was huge difference between control and silica treated plot because at grain filling stage, pollen were washed out due to the high rainfall during 34 to 38 meteorological weak of 2017 but higher percentage of grain filled observed in silica treated plot. In case of, the application of different levels of silica as well as in-

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teraction effect of different levels of nitrogen and silica showed non-significant results regarding straw yield of rice.

Table 7. Effect of different levels of nitrogen and silica on grain and straw yield of rice

Treatment Level	Grain Yield (q ha-1)	Straw Yield (q ha-1)
N0	18.50	34.83
N1	23.02	37.67
N2	25.69	40.38
N3	35.99	48.24
Mean	25.80	40.28
SE	2.22	2.25
CD	6.41	6.49
Si0	19.50	37.49
Si1	24.86	38.73
Si2	27.63	41.21
Si3	31.21	43.69
Mean	25.80	40.28
SE	2.22	2.25
CD	6.41	NS

4. Discussion

The plant height of the rice crop increasing with increasing nitrogen fertilizer levels [16] and it also increased with the age of the crop. It was also observed from this research that, silicon application increases plant height because leaves and stem become more erect, thus reducing self-shading and increasing photosynthesis rate, especially under conditions of high population densities and high dose of nitrogen (Ghanbhari 2011). Analogous verdicts existed informed regarding the response of silica application on rice crop [6,10,26]. This higher nitrogen application which influence obligate augmented the chlorophyll formation and enhanced photosynthesis and there by amplified the plant height and total of tillers per unit area leading to the production of high dry matter. Upturn in nitrogen might have assisted in grander photosynthesis and nitrogen being a basic constituent of protoplasm and chloroplast might have inspired meristematic growth and thus increased the several growth parameters of semi-dry rice [24]. Parallel results were too obtained by the silica application regarding yield of paddy [1,8,20,26]. The application of N and P fertilizers considerably increased total Si uptake by rice [31]. Incorporation of 2, 4 and 6 t CSS ha^{-1} with PU+SSP caused a significant rise in silicon uptake. They likewise observed that total uptake of N, P, Ca and Mg increased by rice as the dose of the CSS increased with N and P. The increase in grain yield influence remain due to more efficient use of solar radiation, moisture and nutrients since silicon makes the rice plant more erect [23].

An experiment was conducted [17] in which it was obtained the various yield due to use of different levels of Si (0, 483, 966, 1448 kg ha^{-1} of Si as calcium silicate) in different soils of Karnataka Mangalore (Typic Kandiustults), Mudigere (Paleustults) and Ponnampet (Ustic Palehumults) ranges from 25.4 to 40.9 Kg pot^{-1} and the rate of increase in yield ranges from 1.1 to 16.0 kg pot^{-1}.

The higher grain yield and straw yield were recorded similar to the finding of [7]. It may be due to higher N rates, which primarily increased the chlorophyll concentration in leaves and there by higher photosynthetic rate and ultimately plenty of photosynthates available during grain development [14].

The grain yield reaction to silicon application could be there in line towards increased leaf erectness, diminished mutual shading affected by dense planting and high nitrogen application, nitrogen upsurges susceptibility to various diseases in rice [26,35]. Silicon fertilizer application decreased blank spikelet number in rice and that created plant not to have enough carbohydrates to fill up all spikelet produced as the silicon fertilization level increased contribution to decline the number of blank spikelet and to surge filled spikelet percentage [7]. Si fertilization increased [1,19] rice straw yield and grain yield [22].

The concentration of nitrogen deteriorated in straw with age and its content was least at harvest. Growth wise alterations in nitrogen content as affected by nitrogen application [13,15,26,36]. The silicon application has potential to elevate optimum nitrogen degrees due to its synergistic effect, silicon fertilized plant might have added maximum benefit of adequate nitrogen available because it reduced harmful effect and stimulated photosynthesis [9].

5. Conclusion

It is concluded that the application of 80 per cent RDN through Konkan Annapurna Briquettes (KAB) with Silica @ 100 kg ha^{-1} which was applied at 7 days after transplanting between four hills was found promising to enhance the rice yield of Ratnagiri-24 variety in lateritic soils of Konkan during *Kharif* Season. The application of nutrients in the form of Konkan Annapurna Briquettes along with the application of silica at a certain level can

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reduce the recommended dose of fertilizer to the extent of 20 per cent during *kharif* season in Konkan region of Maharashtra without hampering the yield level of the same crop.

6. Summary

The research trial was conducted with the interest of the increasing the availability of the nutrients to the rice crop under submerged condition. Because of the lower availability of the nitrogen in lateritic soils of *konkan* region we tried to point out the relation between silica and nitrogen related to the rice crop. The research was completed with the proper aims and objectives with the help of my research guide Dr. N. B. Gokhale, Deputy Director of Research, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli.

Conflict of Interest

We all authors declare that we don't have any conflict of interest.

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ARTICLE

Vermicompost and Cow Dung Admixture Increases Rhizosphere Bacterial Population and Promotes Rapid Physiological Maturity in Maize (*Zea mays* L.)

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ABSTRACT

Incessant application of chemical fertilizers to the agricultural fields may alter the composition and activities of soil microbiota. Thus, the shift of cultivation practices from chemical to organic is considered to be the need of the hour in order to maintain soil health. A study was conducted in the agricultural fields of the University of Burdwan, India to observe the impact of organic manure on the rhizosphere bacterial community. The experiments were conducted on maize plants, supplemented with the recommended dose of chemical fertilizer and organic manure (vermicompost and cow dung mixture). Corresponding changes in the plant phenological events and soil health in terms of soil physico-chemical factors and rhizosphere bacterial groups up to the level of CFU g-1× 105 dry soil was noted. The results showed a significant increase in population of phosphate solubilizing bacteria during 30DAS. However, at 90 DAS, significant increase in the population of phosphate solubilizing bacteria, nitrifying bacteria, asymbiotic nitrogen-fixing bacteria and protein hydrolyzing bacteria was observed in the organically treated plots. The growth of rhizosphere bacteria was attributed to the type of organic manure supplied to the agricultural fields. In addition, a strong correlation was observed between Zn and protein hydrolyzing bacteria. The soil organic carbon and available nitrogen were strongly correlated with nitrifying, fat solubilizing and phosphate solubilizing groups of bacteria.

1. Introduction

The soil health is governed by the physico-chemical factors and the microorganisms present in the soil. The important soil processes such as organic matter decomposition, nutrient cycling and soil degradation are maintained by soil microorganisms [1,2]. The change in the soil microbial community is the most reliable indicator of the soil health of any agroecosystem [3]. For the last two decades, there has been a gradual shift towards the use of organic manures in the agricultural fields, as the manures improve the nutrient availability through soil microbial

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processes and biological interactions [4].

Moreover, the organic manures are rich in organic matter and maintain the soil moisture, thereby improving the soil microbial structure, function and shapes the crop production [5,6]. Wang et al. [7] showed that the addition of organic manures to the soil increases the levels of organic matter, soil porosity, moisture, structural stability and biological activity. The parameters of soil biological processes like the rate of decomposition, nitrification, nitrogen fixation and denitrification are dependent upon the structural stability of the soil microbial community [8,9]. Thus, a better understanding of the effect of organic manures on the soil microbial community could help to reveal the key players of soil biological interactions responsible for crop growth [10].

Here, the study was carried out on maize (*Zea mays* L.) plant. The plants were treated with chemical fertilizer and recommended dose of organic manure. Sangoi [11] studied the effect of plant density on maize yield. The study emphasized on the management practices like water availability, planting date, row spacing and soil fertility, to be maintained in the field for maximum maize production. Subedi and Ma [12] pointed out that the weed infestation is the primary limiting factor of maize yield followed by nutrient (nitrogen) application and plant population density. Milander [13] propounded that the solar irradiation, water availability and temperature are the physical factors that influence the grain yield in maize.

Vermicomposting is a process by which organic materials are converted into vermicompost that is a peat-like material and exhibit high porosity, aeration, drainage, water holding capacity and rich in microbial activity. It is a non-thermophilic and biological oxidation process in association with earthworm and soil microbes [14]. Zhong et al. [15] studied the effect of chemical fertilizer and organic manure on the phospholipid fatty acid profiles and the microbial functional diversity. The work showed that the combination of balanced dose of fertilizer (N, P, K) and organic manure promotes soil microbial biomass and activity. Li et al. [16] showed the application of green manure (*Brassica* sp.) on the soil properties that influence the microbial growth. In Asia, research efforts were made by several authors about application of organic manure and crop growth [17, 18, 19, 20, 21]. Earlier research works were focused on crop performance and productivity. Nevertheless, the underlying role of specific organic manure in application is yet to be understood.

The objective of the present research was to study the impact of chemical fertilizer and organic manure (vermicompost and cow dung mixture) on the rhizosphere bacterial community and the maize phenology.

2. Materials and Methods

2.1 Study Site

Field locations were designed at Crop Research and Seed Multiplication Farm (CRSMF), University of Burdwan, West Bengal, India (latitude, 23°14’58.04”N and 23°15’19.44”N, longitude: 87°50’34.29”E and 87°50’43.95”E). The experiments were done using one variety of *Zea mays* L. (var. *RE-55, Royal England*) during May-August of 2016 and 2017. The seeds were obtained from CRSMF, Burdwan.

2.2 Plot Design and Ttreatment

The field plots of 4 × 3 m^2 were divided under three treatments such as control, chemical and organic. For each treatment, three replications were arranged in a Randomized Block Design (RBD) [22]. The seeds were soaked in distilled water for 24 hours and sown in the control, chemical and organic plots on 26-05-2016 and 30-05-2017. One seed was sown per hill at a depth of 0.03 - 0.04m with row spacing of 0.5m and plant-to-plant spacing of 0.5m. The spacing was done to provide an adequate gap for horizontal expansion of maize roots. Irrigation channels of 0.45m wide were made in between the replications in order to ease the flow of irrigation for each plot. The crops were harvested after the attainment of physiological maturity (R6) stage in the organic plots (05-08-16, 08-08-17), chemical plots (25-08-16, 23-08-17) and control plots (31-08-2016, 03-09-17).

For the hybrid varieties of maize crop, the chemical plots were treated with the recommended dose of *N* in the form of urea at the rate 120 $kg \cdot ha^{-1}$, phosphorus (*P*) in the form of single super phosphate at the rate of 60 $kg \cdot ha^{-1}$ and potassium (*K*) in the form of muriate of potash at the rate of 40 $kg \cdot ha^{-1}$ [23]. At the time of sowing, the fertilizers were applied in such a way that it was not in touch with the seed.

The vermicompost derived from cow dung and processed cow dung were added in the ratio of 1:1 and applied to the organic plots at the rate of 600 g m^{-2} [23]. The combination ratio was accepted and practiced by local farmers, as this combination produced better yields in other crops. No fertilizer or manure was added to the control plots. The maize cultivation was performed for the two consecutive years. The field plots were hand weeded twice, at 20 DAS (days after sowing) and 50 DAS, as the infestation of weeds might cause hindrance in maize growth and procurement of nutrients from the soil. As per the agronomical procedure of maize cultivation, the other fertilizer or manure application was done before the

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attainment of tasseling (VT) stage. The first irrigation was made after the seeds were sown. Later, the frequency of irrigation was maintained as per the requirement by the crops. No crop protection was adopted as the crops did not exhibit any sign of insect/pest attack or disease incidence.

2.3 Soil Sample Collection

Five random sites were selected from a plot for sampling, the soil was sampled through soil sample borer from the rhizosphere zone of maize plants (0.08-0.15m), mixed thoroughly and kept in sterilized polythene bags. The soil samples were collected at V6 stage, VT stage and R6 stage (physiological maturity). The microbial diversity was determined from the soil samples in the Parasitology and Microbiology Research Laboratory, The University of Burdwan.

2.4 Physico-chemical Factors

Physico-chemical variables of soil such as root temperature, T (^oC); available phosphorus, P (kg ha^{-1}); available sulphur, S (mg kg^{-1}); available boron, B (mg kg^{-1}); available zinc, Zn (mg kg^{-1}); available potassium, K (kg ha^{-1}); available nitrogen, N (kg ha^{-1}); organic carbon, OrgC (%); electrical conductivity, EC (μS/cm); soil pH (pH); and soil moisture, M (%) were measured. The T, and other variables like pH, EC and M were determined using temperature meter (LUTRON, PTM-816) and (EUTECH, multiparameter, PM-700) respectively. The data were collected at different vegetative and reproductive stages of the maize plant. Soil available N was measured following the alkaline permanganate method. The available P was estimated following Olsen et al. [24]. The available K was measured by flame photometer after calibration. The water-soluble B was estimated using an azomethine H method [25]. The available S in soil was extracted using monocalcium phosphate and the method described by Bardsley and Lancaster [26]. The available Zn was estimated following Lindsay and Norvell [27]. The OrgC was measured following modified Walkley-Black method [28].

2.5 Rhizosphere Bacteria Analysis

The non-symbiotic bacteria were analysed from the soil suspensions. These include eight groups such as asymbiotic nitrogen-fixing bacteria (ANFix), fat-hydrolysing bacteria (FHydro), heterotrophic bacteria (Het), nitrifying bacteria (Nit), nitrate-reducing bacteria (NRed), phosphate-solubilizing bacteria (PSol), protein hydrolysing bacteria (PHydro) and starch-hydrolysing bacteria (SHydro). The soil samples were kept at 4^oC, where the microbiological analysis was not performed immediately. For the determination of Het group, the soil samples were diluted up to 10^{-3} and a 20 μl soil suspension (10^{-3}) was mixed with 25 ml nutrient agar (peptone 5 g l^{-1}, beef extract 3 g l^{-1}, agar 2 g l^{-1}, pH 7) and incubated at 30 ± 1 ^oC in the BOD incubator. The soil samples were incubated on starch agar media for 24 h for the determination of SHydro group; those bacterial colonies were counted that produced halo zone after flooding with Gram's iodine. After 5-30 days (5 day intervals) from the date of incubation, the bacterial population of Nit group were enumerated on Winogradsky's medium containing $(NH_4)_2SO_4$ (1.0 g l^{-1}) and the colonies were identified (pink colour) by flooding the plates with sulphanilic acid reagent. During the determination of the PSol group, a halo zone formation was observed around the colonies on the insoluble phosphate $[Ca_3(PO_4)_2]$ containing medium. The nitrogen-free medium was used to determine the ANFix group of bacteria. The nitrate agar medium and spirit blue agar medium with TWEEN 20 were used to determine NRed and FHydro groups respectively. These groups of bacterial populations were determined after 1-3 days of incubation [29, 30, 31, 32]. PHydro group of bacteria was determined by the presence of a halo zone around the colonies by flooding with $HgCl_2$ on nutrient agar medium with 2% gelatine.

2.6 Maize Growth Stages

The phenological events of maize plant was divided into vegetative phases; VE: Emergence, V3: Third leaf collar, V6: Sixth leaf collar, V10: Tenth leaf collar, Vn: nth leaf collar visible, VT or Tasseling: last branch of tassel is completely visible; and reproductive phases; R1: Silking, silk visible outside the husk, R2: Blister, kernels are white and resemble as blister in shape, R3: Kernels are yellow with white milky fluid, R4: Dough, milky inner fluid thickens into pasty consistency, R5: Dent, all kernels have dent, R6: Physiological maturity, black abscission layer has formed [33]. These plant phenological events were measured directly from the field. Five replicates were used at each stage, and the average was considered in the present study.

2.7 Statistical Analyses

To understand the association between the bacterial population groups in the organic and chemically treated plots and the physico-chemical factors of soil, principal component analysis (PCA) was done using SPSSv.16 software. To compare the means among the control, organic and chemically treated plots at V6 and R6 stages,

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single-way ANOVA followed by LSD was performed using SPSS v. 16 software.

3. Results

3.1 Rhizosphere Bacterial Community Exhibit Significant Change at R6 Stage of Maize

The improvement in soil health of the maize fields was clearly indicated by the significant rise in population of some of the bacterial groups under study. The ANOVA at R6 stage of maize showed that the Nit, ANFix, PSol and PHydro groups were significantly higher in number at organic plots in comparison to the corresponding chemical and control plots (Figure 1a and 1b), p< 0.05. The LSD result confirmed that no such significant increase in other bacterial groups was observed during the period of study. The Figure 1a and 1b shows the bacterial distribution among control, organic and chemical plots at 30 DAS and 90 DAS respectively.

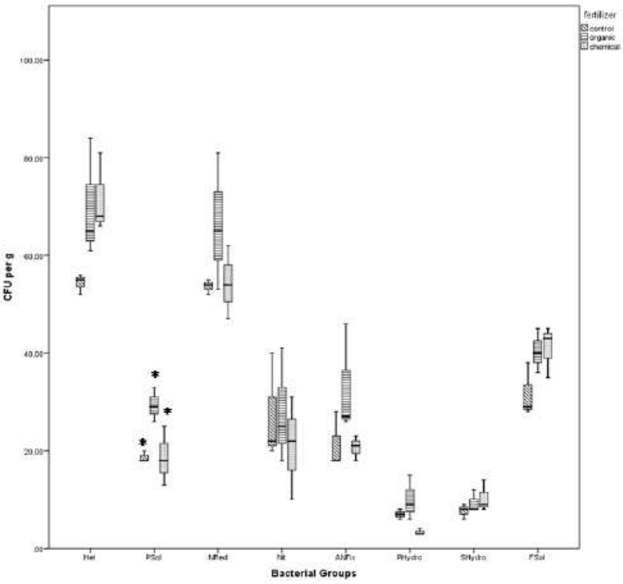

Figure 1a. Box plot showing bacterial population (CFU $g^{-1} \times 10^5$ dry soil) in the control, organic and chemical field plots during 30 Days. Only the phosphate solubilizing bacteria (PSol) showed the significant difference ($p < 0.05$). The (*) over the error bars of box plot represent the significant difference among control, organic and chemical field plots.

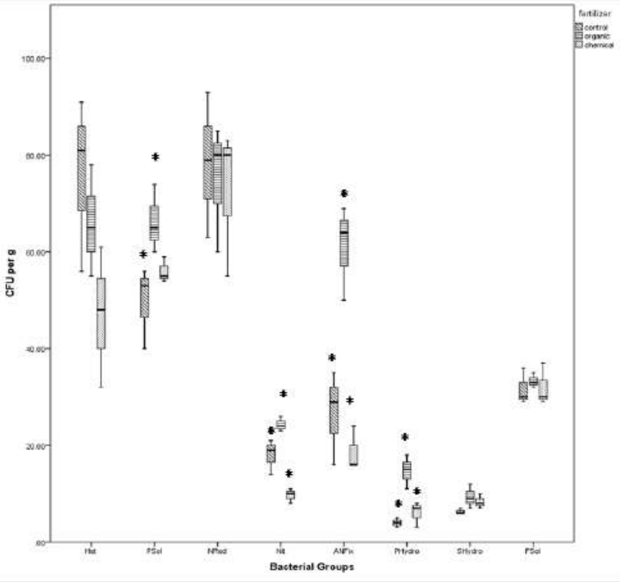

Figure 1b. Box plot showing bacterial population (CFU $g^{-1} \times 10^5$ dry soil) in the control, organic and chemical field plots during 90 DAS. Significant difference ($p < 0.05$) in bacterial population (phosphate solubilizing bacteria (PSol), asymbiotic nitrogen fixing bacteria (ANFix), nitrifying bacteria (Nit), and protein hydrolysing bacteria (PHydro) was observed between organic and chemical field plots. The (*) over the error bars of box plot represent the significant difference among control, organic and chemical field plots.

3.2 Changes in Physico-chemical Factors of Soil

The physico-chemical factors of the soil showed variation among control, chemical and organic field plots. The ANOVA result showed significant difference among the treatment plots. Post-hoc test through LSD showed all the physico-chemical variables except soil pH and available B had significant difference between organically treated plots and chemical plots with respect to control plots ($p < 0.001$). The details of the distribution of physico-chemical factors are shown in Table 1.

Table 1. Physico-chemical factors of soil under organic and chemical treatments, value ± S.E.

Treatments	pH	EC(µS/cm)	T(oC)	M(%)	OrgC(%)	N(Kg/ha)	P(Kg/ha)	K(Kg/ha)	Zn(mg kg-1)	B(mg kg-1)	S(mg kg-1)
Control	5.87 ±0.07	0.078 ±0.005	29.8± 1.49	10-20	0.86 ±0.02	155.4 ±0.68	257.4 ±0.68	609.6 ±0.51	2.40 ±0.01	0.574 ±0.02	18.01± 0.03
Organic	7.12 ±0.03	0.34 ±0.01	28.6± 1.82	35-40	0.96 ±0.01	164 ±0.55	227.8 ±0.66	470 ±0.71	2.54 ±0.01	0.63 ±0.01	16.41 ±0.01
Chemical	5.80 ±0.01	0.22 ±0.01	31.7± 0.85	15-30	0.81 ±0.02	171.2 ±0.37	237.4 ±0.68	551.2 ±0.37	3.058 ±0.03	0.634± 0.05	19.68 ±0.02

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The organic manure used in the organic field plots showed individual variation in NPK and C:N ratio (Table 2). The C:N ratio was higher in cow dung (50.24) than the vermicompost. The higher values of NPK were noted in vermicompost.

Table 2. The NPK (average) of vermicompost and cow dung manures applied to maize fields

Manure	Nitrogen (%)	Phosphorus (%)	Potassium (%)	C:N ratio
Vermicompost	1.92	2.22	1.08	14.09
Cow dung	1.04	0.78	0.15	50.24

3.3 Attainment of Physiological Maturity of Maize Plants

The results of the phenological events of maize plants in the control, chemical and organic treated plots were shown in the Figure 2. Despite the similar environmental conditions, the maize plants in the organic, chemical and control field plots attained the physiological maturity or R6 stage in 86 ± 4.3 days, 95 ± 2.85 days and 107 ± 7.49 days respectively. The average maximum height of maize plants (at tasseling or VT stage) in the organic, chemical and control field plots were found to be 197.3 ± 29.59 cm, 196.38 ± 29.4 cm and 188.98 ±22.6 cm respectively. In control and chemically treated field plots, it was observed that there was a delay in the progression from one vegetative or reproductive stage to the next succeeding stage.

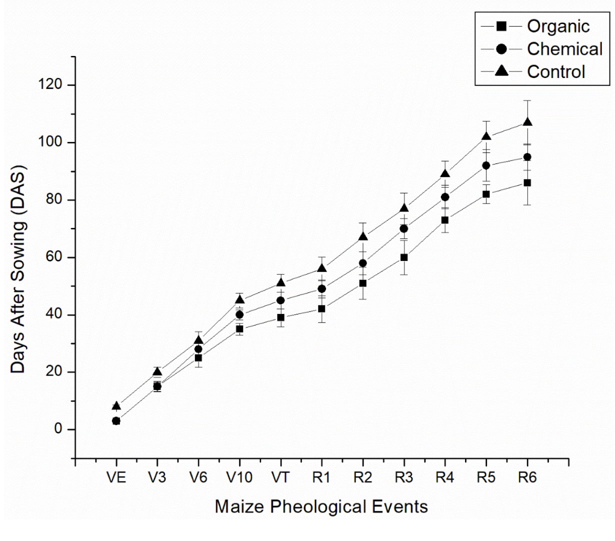

Figure 2. The appearance of phenological events of maize plant (VE: Emergence, V3: Third leaf collar, V6: Sixth leaf collar, V10: Tenth leaf collar, VT or Tasseling: last branch of tassel is completely visible; and reproductive phases; R1: Silking, R2: Blister, R3: Kernels are yellow with white milky fluid, R4: Dough, R5: Dent, and R6: Physiological maturity) in the organic, chemical and control field plots. The bars on the line graph represent the standard deviation from the mean.

3.4 Association of Rhizosphere Bacteria and Physico-chemical Factors

The PCA results of chemical and organically treated plots are shown in Figure 3 and Figure 4 respectively. In the chemical plots, factor 1 accounts for 50.13% and factor 2 accounts for 30.67 % of the variables (Figure 3). The physico-chemical factors like P, Zn, B, S, OrgC formed a close association with PSol and ANFix. On the other side, EC formed a cluster with SHydro, PHydro and FSol. A close association between soil pH and K was observed when factor 2 was considered.

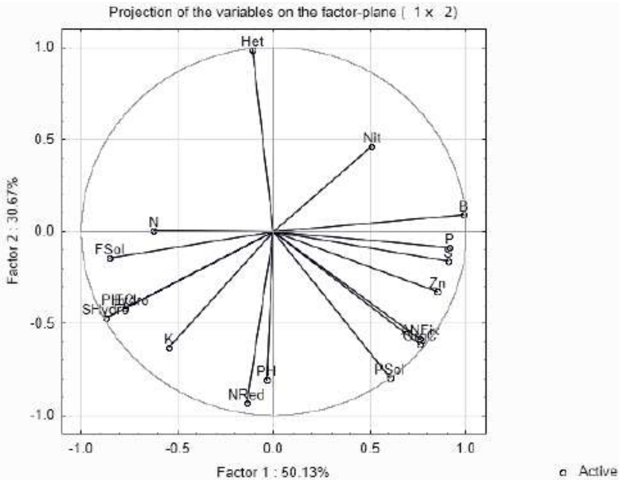

Figure 3. The PCA biplot of the chemical field plots showing the association of rhizosphere bacterial groups and physico-chemical factors.

In the organic field plots, factor 1 and factor 2 explained 46.32% and 28.39% of the variables respectively (Figure 4). A strong correlation was observed among PHydro, SHydro and Zn. Another cluster was formed by soil pH, P, K and B that appeared opposite to the above bacterial groups. The soil properties like OrgC and N were strongly correlated with Nit, FSol and PSol.

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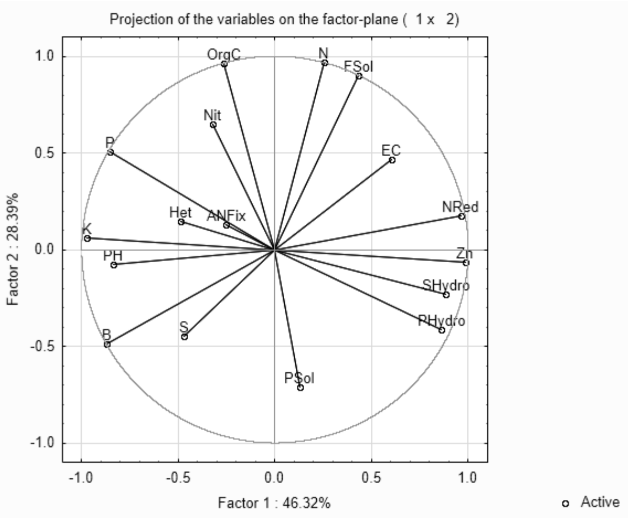

Figure 4. The PCA biplot of the organic field plots showing the association of rhizosphere bacterial groups and physico-chemical factors

4. Discussion

The roots are the indicator of soil health [34]. The maize plant treated with chemical fertilizer (NPK) and organic manure (vermicompost and cow dung) showed a remarkable difference in the growth events as well as in the soil bacterial composition. The significant increase in the Nit, ANFix, PSol and PHydro group of bacteria in the organic field plots at R6 stage were attributed to the application of recommended dose of vermicompost and cow dung composition. Alidadi et al. [35] experimented with the application of vermicompost and cow dung on tomato plants and concluded that the vermicompost enriches the soil with essential nutrients. Similar results were found by Karmakar et al. [36], while experimenting on rice plant. According to Fulhage [37], organic manure consists of the three major plant nutrients, nitrogen, phosphorus and potassium (NPK), and the essential nutrients such as Ca, Mg, S, Zn, B, Cu, Mn *etc.* for maize growth.

Adegunloye et al. [38] showed that the C:N ratio in cow dung manure is the indicator of protein that serves as an important protein source for the microbes which involved in the decomposition of organic matter. The organic manure raises the pH level of soil and accelerates the decomposition of organic matter and termite activity. The present study indicated that the population of the protein hydrolyzing bacterial group (PHydro) was significantly higher in the organically treated plots at R6 stage. Ram [39] studied the bacterial strains in cow dung responsible for plant growth-promoting traits. The study showed that the cow dung necessarily contains the bacterial strains that are responsible for Indole Acetic acid production (IAA) and phosphate solubilisation.

Atiyeh et al. [14] showed that the nitrification process in soil, resulting in the rapid conversion of ammonium-nitrogen to nitrate nitrogen are favoured by vermicompost along with cow dung manure and this nitrate nitrogen is crucial for maize or other plant growth. Besides, they also observed the increase of nitrate-nitrogen to 28 folds after 17 weeks of DAS, while in conventional compost, this increase was only 3 folds. The increase in the ash content showed rapid mineralization or organic matter. The ash hinders the H_2S formation and improves the availability of O_2, thus help the nutrient uptake by maize plants. The high phosphorus (%) was due to mineralization and mobilization of phosphorus resulting from the enhanced phosphatase activity by the microbes present in the gut epithelium of earthworm [40, 41]. The present study corroborates the above findings.

Plant growth promoting (PGP) microorganisms including PGP-Rhizobacteria (PGPR), phosphate solubilizing microorganisms (PSMs) and other symbiotic microorganisms like arbuscular mycorrhizas (AM) fungi, may play a major role in developing a sustainable use of P resources and making them solubilise in the soil [42]. The potential role of phosphate solubilizing bacteria (PSol) and P availability of vermicompost have been studied by several authors [43, 44, 45]. Their results have showed the increase P availability to plants through phosphate solubilization by PSol. Bhatt and Maheshwari [46] advocated that the cow dung consists of phosphate solubilizing bacteria that help in the early growth of plants. Moreover, the study highlighted the bacteria groups present in the cow dung promote reproductive growth in plants. The gradual increase in the PSol at 30 DAS through 90DAS in the organic plots and subsequent maize growth was attributed to the manure mixture supplied to the plants.

The vermicompost and cow dung composition not only enhances the population of free-living nitrogen fixers, but also promotes the plant growth through nitrification, phosphate solubilisation, and plant disease suppression [47,48]. Sinha et al. [49] added that this process is possible due to complex interactions between earthworm and plant growth-promoting microbes. The earthworms ingest plant growth-promoting rhizobacteria like Pseudomonas, Bacillus, Azosprillium, Azotobacter etc. along with rhizosphere soil, and they might get activated or increased due to the ideal micro-environment of the gut. When released into the environment, these microbes serve as a plant growth promoter and help the maize plant in rapid growth attainment. In the present study, the increased population of the ANFix group and Nit group of bacteria, observed in the organic plots were probably due to the above reasons.

The previous studies on the effect of vermicompost on soil pH, EC and C:N ratio showed that the earthworm activity reduces pH and C:N ratio in manure [14] This slightly decreased value of pH is due to mineralization of N and P, microbial decomposition of organic material into the intermediate organic substances like humic and fulvic acids [10] and simultaneous evolution of CO_2 [40]. However, the values of the soil pH of the organic field plots were a bit higher than the chemical counterparts. Soil pH is considered to be a significant factor contributing to the structure of soil microbial community [50]. Wu et al. [51] showed that the increase in pH of soil caused shifts in the microbial community. Li et al. [52] propounded that the addition of organic manure increases the soil pH, which in turn increases the soil organic matter, promotes soil maturation, and enhances the soil base saturation percentage. The present study showed that the average soil pH values were higher in organic plots in comparison to chemical plots.

The strong correlation between the Zn and PHydro group of bacteria in the soil of organic plots might be the reason for the accelerated growth of maize plants. Protein hydrolysis through PHydro releases a mixture of peptides and amino acids that are adsorbed by the roots and then translocated to the plant [53]. Ertani et al. [54] reported that the protein hydrolysates have a positive effect on plant root and shoot growth in maize plants. The PHydro present in the organic manure consists of biostimulants like IAA that promotes plant growth [55]. Zn has an essential role in the synthesis of tryptophan, which is considered to be the precursor of IAA [56]. The present study showed a strong correlation between Zn and PHydro. Besides, protein hydrolysates promote the nitrogen assimilation in plants through co-ordinated regulation between C and N metabolism [55]. Schiavon et al. [57] showed that the protein hydrolysates enhanced shoot biomass production in maize plants. They found that the biostimulants increase the activities of the nitrate reductase enzyme. In the present study, the PCA results of the organic plots showed that the PHydro and NRed groups of bacteria were under the same cluster. In the organic plots, the addition of vermicompost and cow dung mixture increased the organic carbon content, which in turn increased the moisture content and aeration. As a result, the nitrification process was promoted in the soil. Nie et al. [58] studied on the N_2O emission from the soil about soil carbon and nitrogen content and found similar results.

The chemical plots indicate strong correlation between available P and PSol group of bacteria. Studies on phosphate solubilization in soil showed that the bioavailability of P is regulated by the activity of PSol. The PSol group of bacteria converts nonlabile P through either inorganic P-solubilizing or organic P mineralization processes [59]. The study also revealed that the ANFix group of bacteria was closely associated with OrgC. Roper and Gupta [60] inferred that the non-symbiotic nitrogen fixing bacteria are largely dependent upon the carbon stock of the soil, as the organic carbon resources are essential for their enzyme activity. The present findings were in agreement with the above statement.

5. Conclusions

The present study revealed some interesting findings. The application of vermicompost and cow dung mixture promotes maize growth with significant increase in the four groups of rhizosphere bacteria like PSol, Nit, ANFix and PHydro. This clearly indicates that the process of phosphate solubilization, nitrification, nitrogenase activity and protein hydrolysis are enhanced by the application of organic manure used in the experiment. The protein hydrolysates released by the action of PHydro in the organic manure help in the release of biostimulants like IAA, which in turn increases the maize plant growth. Organic carbon associated with the organic manure is considered as the essential sites for microbial activity. The application of the recommended combination (1:1) of vermicompost and cow dung could be suggested to the farmers to raise maize production in the tropics. Thus, it can be said that the present combination of organic manure not only maintains soil health but also promotes sustainable agriculture.

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