GREEN SYNTHESIS OF COMMON SILICA AND NANO SILICA AND THEIR IMPACT ON LEAD TOXICITY IN WHEAT

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LIST OF ABBREVIATIONS

EC	Electrical Conductivity
EL	Electrolyte leakage
MDA	Malondialdehyde
ROS	Reactive Oxygen Species
RH	Rice Husk
RHA	Rice Husk Ash
SEM	Scanning Electron Microscope
XRD	X-ray Diffraction

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ABSTRACT

Lead (Pb) is an unimprtant toxic heavy metal that decreases agricultural productivity, by impacting the physiological and biochemical properties of various crops. Lead toxicity causes wheat productivity to plunder, by inducing oxidative stress in wheat. Wheat is an essential part of human diet. It is a nesessary food for 50% of the global population. Silicon is known to counter the adverse effcts of heavy metal toxicity. The aim of this study was to synthesize common silica and nanosilica from rice husk and, to observe their effect on wheat grown under lead contaminated soil. Rice husk was leached with hydrochloric acid, and then rinsed with de-ionized water and after heating it in muffle furnace at 700 °C, silica particles of nano size were obtained. Different heating cycles were employed on rice husk to synthesize common silica particles. Wheat was grown in pots containing 5 kg of lead contaminated soil to observe the impacts of silica nanoparticles. Three different levels of common silica and nano-silica particles (0, 300 and 500 mg/kg) were applied in the soil before one week of wheat sowing. The lowest chlorophyll content, root weight, shoot weight and husk weight were observed in the control plants while the highest lead concentrations in shoots, roots, and grains were observed in the control plants. Common silica and nano-silica particles improved wheat growth by increasing root, shoot and grain weight, and counter oxidative stress in wheat by lowering hydrogen peroxide, electrolyte leakage and malondialdehyde content in leaves. When compared with control, nano-silica treatment reduced Pb concentration in wheat root, shoot and grain. This study indicates that silica treatment improves the growth of wheat in a lead contaminated soil by restricting its entry into wheat. NPs silica are more efficient in countering heavy metal toxicity in wheat than bulk silica particles.

Keywords: wheat, nano silica, growth, oxidative stress, sustainability

Chapter 1

Introduction

1.1 Background

Wheat is quite significant crop from economic perspective, and its contribution to human diet is unparalleled. Annual wheat production was about 680 million metric tons in 2012, since then its production is showing an upward trend reaching 750 million metric tons in 2020. After maize and rice, wheat ranked 3rd in terms of providing food for global population. (Shewry and Hey 2015). At any stage, human can't afford a significant drop in wheat production because human diet is heavily dependent on wheat.

Wheat is a quite resilient crop, it has potential to grow in diverse climatic conditions, but still there are certain factors that affect the wheat growth and productivity, like soil conditions, crop management techniques, etc. It is predicted that human population would increase by two billion people in 2050, therefore it is of utmost importance to increase food production. Wheat varieties that are resistant to heat and drought must be prioritized in this regard to meet the food requirements (Asseng et al. 2015). (Wieser et al. 2020) reported that chemical composition of wheat grain is variable, it depends upon type of wheat specie, variety, and climatic condition. However, its composition varies in a small range, keeping in view the range chemical composition of wheat grain is discussed here

Table 1.1 Composition of wheat grains

Constituents	Composition
Water	13%
Starch	58%
Non-starch carbohydrates	13%
Proteins	11%
Lipids	2%
Vitamin	0.10%
Minerals	2%

1.2 Silicon

Silicon dioxide is an important rock forming material which is present in metamorphic and magmatic rocks. It is an integral component of earth's crust. It's abundance in earth's crust can't be ignored, since it accounts for 27.7% of the earth's crust by weight (Greve 2012).

Silicon is not regarded as necessary chemical for plant, but its deficiency poses serious problem for plant growth. Silicon proves beneficial when plant is under any abiotic stress (salinity, water deficiency, heavy metal toxicity). Silicon provides vigor and strength to plants which act as a defense mechanism against any stress. Initially, it was thought that silicon provides physical barrier to plants along with cell wall, however, now it was proved that defense mechanism of silicon is far more complex than just providing physical barrier (Luyckx et al. 2017).

(Epstein 2017) reported that Silicon availability is critical for plant growth. Numerous functions of plants get disturbed in the absence of silicon, for example crops are unable to handle abiotic and biotic stress, its growth is compromised, reproductive mechanism also get a hit.

1.3 Phytoliths

Plants absorb silica from soil as silicic acid and then precipitated it as amorphous silica in solid form. There are specialized tissues found in grasses and plants which deposit silica in stem, roots, and leaves. These silica accumulations occur in various shapes and forms, which are known as phytolith, plant phytolith, biogenic silica. These phytoliths are resistant to degradation, so they served as fossils which are used for reconstructing past (Nawaz et al. 2019)

At the end of each growing season, plant organic matter decays, but silica phytolith accumulated in the underlying component of soil. It has found that silica phytolith occurred in almost all ecosystems from rain forest to grass land to wetland ecosystems (Norris and Hackney 1999).

1.4 Silicon fertilizers

Several studies have shown that silicon enhances soil nutrient utilization mechanism by plants and microbes. It was reported that silicon application in the range of 10.4-15.6 μg/kg increases grain yield and crop productivity, but excessive application of silicon proves detrimental for plants. Silicon application can increase utilization of soil nitrogen (Liang et al. 2003).

Soil acidity poses a major challenge in agriculture sector, this problem is remediated using lime (Calcium carbonate) in many parts of the world. One plausible remedy to tackle the problem of soil acidity is the use of silicon fertilizers using calcium and magnesium silicates. Silicon mechanism of action in soil is similar to those of calcium and magnesium, silicon induces chemical reaction similar to those of calcium and magnesium, increasing the soil pH and precipitates toxic metal ions (Marafon and Endres 2013).

1.5 Rice husk

Around 50% of the global population considered rice as a staple food. Seventy nations around the world produce rice including China, Indonesia. Rice husk is an agricultural residue which exists in plentiful quantity in fields. Basically, rice husk is the hard protective coating of rice which is removed from rice seed during milling (Kumar et al. 2013). Existing literature shows that form every ton of rice approximately 0.23 tons of rice husk is obtained. Rice husk being an agricultural waste creates waste disposal problem. If not handled in an appropriate way it creates problematic scenario; as it attracts disease causing animals, it occupies large pieces of land, there is always chance of rice husk catching fire (Souza et al. 2002)

Rice husk has numerous advantages depending upon its chemical and physical properties like its ash content, amount of silica content in it. Rice husk has both domestic applications as well as industrial applications. Some of them are mentioned here:

a. Rice husk is used as fuel in industries. Combustion and gasification generate heat which can be for low-capacity boilers. For generating 1 MWH (Million-watt hour) of electricity, about 1 ton of rice husk is being used.
b. Activated carbon can be obtained from rice husk. Rice husk contains enough cellulose, hemicellulose, and lignin content; therefore, rice husk is used as a basic substance for synthesis of activated carbon. Activated carbon has a quite complex microporous structure hence it is considered as an efficient adsorbent.
c. Rice husk can be used in construction industry as it is used to make bricks. Bricks would be more porous if they contain more rice husk. More porosity leads to better thermal insulation.

d. Rice husk is used as a precursor in synthesis of silica and silicon like compounds. Rice husk contains 20% silica, numerous useful silicon like compounds can be obtained from rice husk like silicon nitride, pure silicon, zeolite, silicon carbide and quartz.

e. Various essential chemicals are derived from rice husk like acetic acid, xylitol, furfural, and ethanol (Zou and Yang 2019).

Table 1.2 Composition of rice husk

Chemicals	Composition
Cellulose	31%
Hemicellulose	22%
Lignin	22%
Mineral ash	14%
Water	7%
Extractives	3%
(Kumar et al. 2010)	

1.6 Rice husk ash

Rice husk ash is genetic term encompassing all types of ash generated during burning of rice husk. When RH undergoes combustion, it generates about 17%-20% RHA. RHA is a lightweight, bulky material whose density is in the range of 180-200 kg/m^3. Two types of ashes are produced with RH, White ash, and black ash. RH undergoing controlled combustion yields white rice husk ash, when it undergoes controlled pyrolysis it yields black rice husk ash – RHA (Zou and Yang 2019). Physio-chemical characteristics of RHA are altered by many factors such as rate of heating, geographical location etc.

RHA is rich in silica content, and no other plant can hold such large quantity of silica, but there are trace elements which are found in ash like sodium, copper, etc. These metallic impurities are linked to soil type, climatic conditions and the type and quantity of fertilizer used during rice growth (Della et al. 2002).

1.7 Silica from rice husk

Amorphous silica is an industrial product, it is quite extensively used in various industries. Synthesizing silica using conventional method proves to be quite expensive. Hence rice husk is used as a precursor to synthesize silica particles because RH is rich in silica content. Two methods are proposed to extract silica from rice husk, i.e., direct combustion method and chemical method.

1.7.1 Direct combustion method

Direct combustion is an old method of generating energy from biomass. During this process agricultural residue are burnt in an open atmosphere releasing energy which can be used for various purposes. Heat released during this process can be used to generate electricity. After burning rice husk ash (20% by weight) is generated which is known as rice husk ash. Rice husk ash contain >90% silica content (Delivand et al. 2011).

During the acidic treatment process rice husk is first incinerated to obtain rice husk ash and then it is treated with a strong acid to obtain pure silica particles (Della et al. 2002).

1.7.2 Chemical method

Silica can be obtained from rice husk via extraction process. During the extraction process rice husk is treated either with a strong acid or with a strong base. It has been reported that solubility of silica increases rapidly by increasing pH, therefore extracting silica from rice husk using alkaline solvent is preferred. (Setyawan et al. 2019).

1.8 Applications of rice husk silica

Rice husk ash is rich in silica content ranging from (83%-90%). This amorphous silica has various applications in numerous industries. It can be used for power generation, as a zeolite, in manufacturing of silica gel, silicon chips and synthesis of activated carbon (Pode 2016). Some of the applications of rice husk silica are presented here:

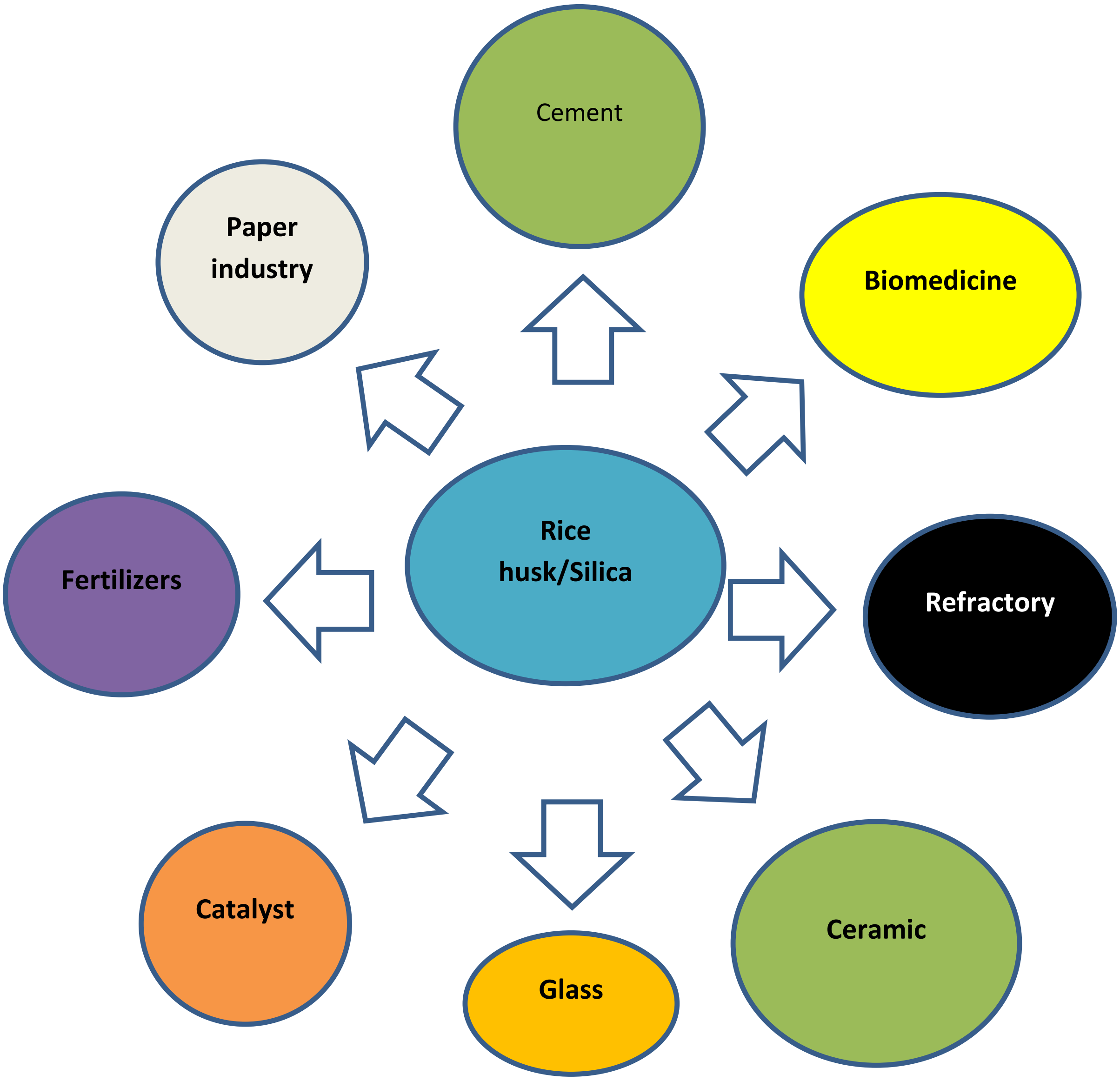

Fig 1.1: Applications of rice husk

Being a toxic chemical, lead has toxic impacts on wheat physiology and biochemistry. Silica is known to tackle abiotic stress in plants, but it has not been applied on wheat to counter the toxic impact of lead. Hence this study is conducted to observe the negative physiological impacts lead have on wheat and how silica can meet this challenge of lead toxicity.

1.9 Objectives

After thorough examination of previous studies, it was hypothesized that silica particles of nano- and normal size can be synthesized from rice husk using eco-friendly processes, and these particles have the potential to mitigate lead toxicity in wheat. Hence, the objectives of this study were:

1. To synthesize nano silica and common silica from rice husk and their characterization.
2. To investigate physiological response of wheat grown on a lead contaminated soil.
3. To compare the efficiency of nano silica and common silica in tackling lead toxicity.

1.10 Significance of the study

Excessive use of fertilizers and mining activities released large quantity of lead into the environment. Lead would ultimately enter food items like wheat. On one hand, this heavy metal is decreasing the yield of wheat by inducing oxidative stress, and on the other hand toxic lead enters food chain and eventually taken up by humans causing health impacts of great concern.

This research focused on how agricultural residue (rice husk) can be utilized in an effective way and, the useful substance like silica can be obtained from this. Moreover, the potential of common silica and nano silica in tackling lead toxicity could be evaluated via this research.

1.11 Scope of the study

This study would be quite helpful in agricultural sector. Wheat is a staple food of over 50% of population around the world, and this research would partly assist in dealing with food security issues. This research would be useful in protecting the environment from toxic lead, by limiting its entry into the food chain.

Chapter 2

Literature Review

2.1 Sources of lead

Lead is a potent heavy metal that has gained considerable attention due to its toxic impacts on soil and water. Lead enters the environment from various sources ranging from petroleum to paper industry. Some of the major sources from where lead gets entry into environment are mentioned here:

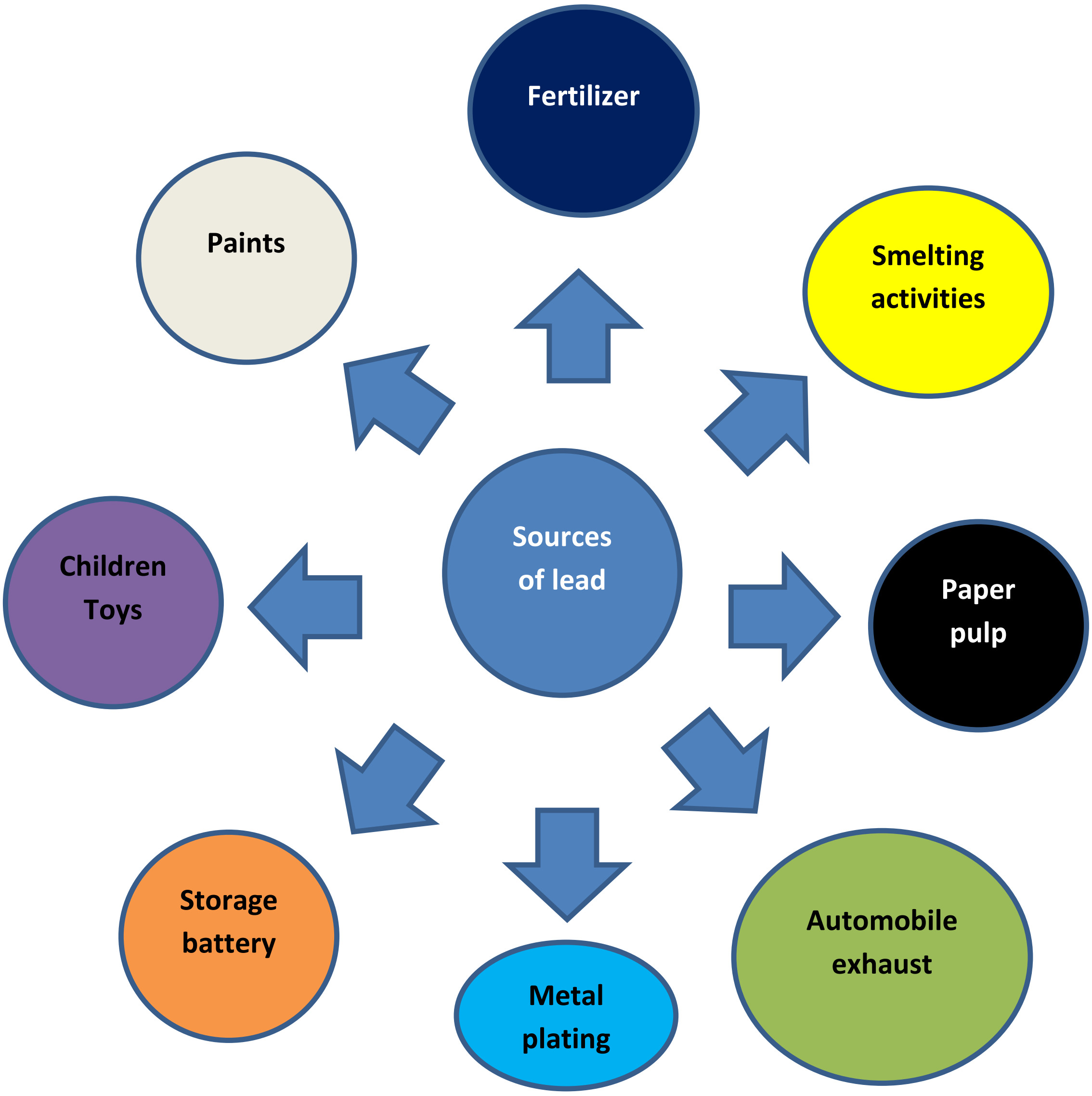

Fig 2.1: Sources of lead in the environment

2.2 Lead impacts on human

Lead contributes about 0.6% of the global incidence of illness, and this effect is more pronounced in developing nations. Approximately 143,000 lead related deaths occurred annually, and it is responsible for about 600,000 mental illnesses around the world. Lead can affect almost every major organ of human body, but its impacts are more significant on kidneys, liver and on neurological systems (Fatmi et al. 2017).

2.3 Lead toxicity in plants

Lead enters plant via roots, therefore maximum concentration of lead is found in the roots of a plant in an insoluble form. Lead accumulation in plants depends upon the external surrounding environment of plants, higher exogenous lead condition leads to a greater level of lead in plant body. Once lead enters plant it can disrupt the various biochemical and physiological function by altering seed germination process, lowering water status. However, lead mobility from roots to shoot is somewhat restricted, but lead has the potential to severely hamper the photosynthetic processes, and carotenoid pigments in plants (Lamhamdi et al. 2013).

2.4 Toxic impacts of lead on soil

Lead has been categorized as hazardous heavy metals, due to its toxic impacts. Long term exposure of lead (even at low concentration) leads to dangerous health and ecological impacts. Numerous adverse impacts of lead are reported on soil health, lead tends to decrease soil fertility, lead reduce essential soil nutrients, which in turn alter soil microbial community particularly in rhizospheric region. Lead affects the floral and faunal community in soil indirectly, but in some cases, lead has its direct lethal impacts, which are detrimental for soil ecosystem. Lead toxicity in soil can increase the mortality of earthworm. Lead can negatively affect soil sorption capacity and tend to decrease humic acid content. Lead toxicity can cause reduction in the microbial and actinomycetal species in soil (Ahmed et al. 2021).

2.5 Lead impact on wheat

Wheat grown in a lead contaminated soil shows a marked decrease in the growth parameters. This decrease in growth can be attributed to lead toxicity, which alter normal physiological and biochemical processes like respiration, electrolyte balance etc. It was reported that lead toxicity in wheat cause a drop in essential nutrient like potassium phosphorus, and the reason for this decline is that Lead can damage the root absorption sites, altering the potential of roots to take up essential nutrients. Rubisco is one of the most important enzymes that take part in carbon fixation process. Wheat subjected to heavy metal toxicity significantly reduces rubisco level (Alamri et al. 2018).

2.6 Nanotechnology

Nanotechnology has gained much attention in the 21st century, as this technology allows scientist to play with complex chemicals at a very small scale (nanoscale). Scientists can alter the various morphological and physiological properties of material, by reducing their size and use these particles for various useful purposes. Nanotechnology has offered numerous advantages in various disciplines, but its benefits are more obvious in the field of biology. Nanoscale particles are basically of the same size as basic components of a biological systems are like organelle, cell and tissues, etc. (McNeil 2005).

2.7 Nano-fertilizers

Nanotechnology is quite useful in agriculture sector. It intends to enhance crop productivity on one hand, and on the other hand it tends to bog down the excessive usage of pesticide and fertilizer application and prove instrumental in tackling environmental pollution. Nanoparticles prove to be quite effective in promoting crop growth and tackling phytotoxicity. Nanoparticles of iron oxide, zinc oxide, alumina, silica are reported to have a positive effect on various edible crops (Rose et al. 2015).

Nanoparticles of silver can induce growth of wheat and enhance its productivity. Nano-particles in agriculture can be used as nano insecticides, as an antimicrobial agent, as nano fungicides, and for controlling plant viral diseases (Achparaki et al. 2012). Nano particles of zinc oxide and iron are proved very useful in tackling cadmium toxicity in wheat. Cadmium was unable to translocate from roots to the grains in the presence of these nano-particles (Rizwan et al. 2019). Nano-silica application enhanced rice tolerance to lead toxicity by activating anti-oxidative mechanism. Nano-silica greatly reduced lead translocation factor in rice by lowering its translocation from roots to shoot, and from shoot to grain (Liu et al. 2015)

Nanotechnology proves to be a useful discipline in the field of agriculture. Nanoscale materials are not only efficient in enhancing the productivity of essential crops, but they are also considered as environmentally friendly, because they rub off the hectic process of managing, reducing, and controlling environmental pollution in a sustainable way. Some of the applications of nanotechnology in agriculture sector are mentioned here:

2.7.1 Nano sized carrier

Fertilizers, pesticides, herbicides can be applied at the nano level. These nano sized chemicals are considered stable against degradation hence they are applied in minute quantity in comparison to conventional fertilizers or chemicals.

2.7.2 Biodegradation of resistant pesticides

Resistant pesticides pose a major problem, because they remain in the environment for a longer period, contributing to the environmental pollution. Nanoparticles tend to convert these harmful pesticides into harmless chemicals, which can easily be decomposed.

2.7.3 Applications of nano-barcodes

Nano barcodes can be used in non-biological applications. Nano-barcodes are uniquely identifiable codes that are used for authentication or tracking of agricultural food product (Gaheen and Hinkal 2012).

2.8 Uses of nano-silica in agriculture

Nano silica plays a prominent role in water deficit wheat and increases average grain number per spike in a limited irrigation regime. Nano-silica is more efficient for wheat growth than other nano-fertilizers like nano boron and zinc oxide, etc. (Ahmadian, et al. 2021). Nano silica is not only good for physiological parameters of wheat, but it tends to activate certain defensive mechanism in case of stress. Antioxidative mechanism speed up under silica treatment, hence any stressful condition can be dealt with the application of silica (Hajihashemi and Kazemi 2022).

Nano silica has a profound impact on wheat growth, it led to increased wheat growth and a marked improvement in it grain weight. Nano-silica can neutralize the toxic impact of heavy metal stress by reducing oxidative stress in wheat (Khan et al. 2020).

2.9 Silicon

Silicon protects plant from harmful ultraviolet radiations by forming silica deposits on leaf epidermis. Silicon also maintains water status in plants. In water stressed environment silicon protect water loss by forming silica-cuticle double layer beneath the leaf epidermis which controls water loss through transpiration. Improved water status in plants under drought stress can be justified by the fact that silicon promotes root elongation which extract more water from the soil. In a metal contaminated soil, silicon decreases bioavailability of heavy metal in plants by raising soil pH (Luyckx et al. 2017).

In case of heavy metal stress, silicon induces plants to initiate certain mechanisms which prove very helpful in mitigating the toxic effect of that stress via:

- Scavenging of reactive oxygen species by provoking antioxidant enzymatic activity.
- Immobilization of toxic metal ions in biologically less active part.
- Enhancing nutrient uptake, and water mobility.

2.10 Silicon importance for plants

Silicon is not categorized as an essential element for plant; however, evidence suggests that silicon proves quite consequential when plants encounter any stress. Silicon has the prospect to counter the negative impacts of abiotic stress like drought, salinity, and heavy metal toxicity, and enhance plant tolerance to these abiotic stresses. Initially, it was thought that silicon protects plants by providing additional physical protection along with cell wall and minimizes nutritional loss in plants. Now it is clear that protective role of silicon is far more complicated which involves several complicated mechanisms (Luyckx et al. 2017). Silicon protects plants from harmful effects of UV radiations by preventing membrane damages (Shen et al. 2010). (Zhu and Gong 2014) reported that silicon maintains water status in drought treated plants, by forming a silicon cuticle double layer beneath the leaf epidermis, which inhabit water loss. Silica tackles saline toxicity by inhabiting the uptake of sodium and chloride ions. Unnecessary translocation of these toxic ions is prevented by silica (Shi et al. 2013).

Silicon is a beneficial plant nutrient; its deficiency causes problematic scenarios for various crops particularly for silica hyperaccumulator crops like rice and sugarcane. Silica application increases yield of crops, this increase can be attributed to the solid defense mechanism of silicon. Silicon tends to tackle abiotic stress in crops by strengthening tolerance mechanism is plants. Crops grown in field experience various stresses like heavy metal toxicity, hyper salinity so silicon fertilizer proves quite effective in tackling these stresses. In the presence of silica, 50% increase in yield are reported in sugarcane in Hawaii, Australia, Florida, and Brazil. Similarly, 1%-4% of yield increase was reported in Japan under silicon application. Various other crops like cucumber, garlic, onion, and potato also respond positively with silicon. Silicon concentration in different crops varies according to the species. However, major differences among the genotype of same species have also been reported (Crooks and Prentice 2017). Silicon improves drought tolerance in crops, it also wilting in certain plants, it minimizes water loss from plants. Silicon helps plant grow under water deficit condition, under saline stress and under heavy metal toxicity.

Silicon induces plant root to release those root exudates, which limit the ability of heavy metal to enter plant root. Silicon accumulates in lignin component cell wall of plants and bind the metal ion along with it, lowering its translocation from roots to the aerial part. Beneficial effects of silicon are more pronounced in the cell wall of roots than in shoots, which stabilizes plant tissues against metallic toxicity. In case heavy metal gets entry into plants, silicon manages this toxicity by storing heavy metal in biologically less active part of plant and restricting its mobility. Silicon enhances nutrient uptake in plant by promoting root growth which absorb more water and nutrients from the soil maintaining nutrient level in plant (Emamverdian et al. 2018).

2.11 Reactive oxygen species

ROS are unstable, reactive oxygen rich unstable radicals that possess unpaired valanced shell electrons i.e., hydroxyl group, peroxyl group and hydrogen peroxide (Wang et al. 2010).

Initially it was thought that reactive oxygen species are a toxic by-product of aerobic metabolism, but with the passage of time it has been proved that these species have some role in plant growth and development, and their role became more prominent in the presence of external stimulus. ROS are produced in different parts of cell like mitochondria, chloroplast, and peroxisomes. ROS have a dual role in cell: they are critical in managing various physiological functions and they also cause oxidative damage to plants under stressful circumstances. There is a delicate balance exists between ROS generation and their scavenging. This balance is vital for normal homeostasis in plant, imbalance led to oxidative stress in plants (Das and Roychoudhury 2014).

ROS are being synthesized outside cell during seed maturation, during photosynthesis and respiratory metabolism. Initially superoxide anion radical is formed from simple oxygen molecule, this unstable superoxide radical undergoes many reactions within cell forming hydroxyl, peroxide, and other reactive oxygen species. Superoxide is a negatively charged radical which is unable to cross cell membrane. Hydrogen peroxide is one of the reactive oxygen species containing no charge and it can diffuse across cell membrane and act as a signaling molecule in defense response (Grene 2002).

Plants produce ROS naturally during unstressed conditions, but under stressful conditions plant synthesis ROS in excess quantity, which became quite problematic for plants. During non-stressed ambient conditions oxygen molecule is reduced to water, in the meanwhile hydroxyl or hydrogen peroxide might be produced. Normal oxygen molecule in plant acquires one electron which generates superoxide anion. Superoxide anion is believed to the initiator of more ROS (Karuppanapandian et al. 2011).

In the stressful environment, quantities of ROS increases manifold, causing disruption in normal functioning of plant metabolic activities. Plants encounter series of imbalances in its normal functioning when excess ROS are produced, hence leading to various physiological and biochemical alteration (Sarker and Oba 2018).

Each ROS has certain peculiar characteristics which are unique to them, for example singlet oxide molecules oxidize lipid and proteins. Superoxide is a ROS with a half-life of about 1-4 μs and reacts with protein content within cell. Hydroxyl radical is an extremely reactive ROS with a half-life of about 1 ns. Hydrogen peroxide is a more prominent reactive oxygen species with a half-life of about 1 ms (Mhamdi and Breusegem 2018).

Superoxide dismutase is an antioxidative enzyme which provides first line defense against reactive oxygen species. Superoxide dismutase convert superoxide radical into hydrogen peroxide, which on further action of catalase converted into water and oxygen molecule (Bowler et al. 1994).

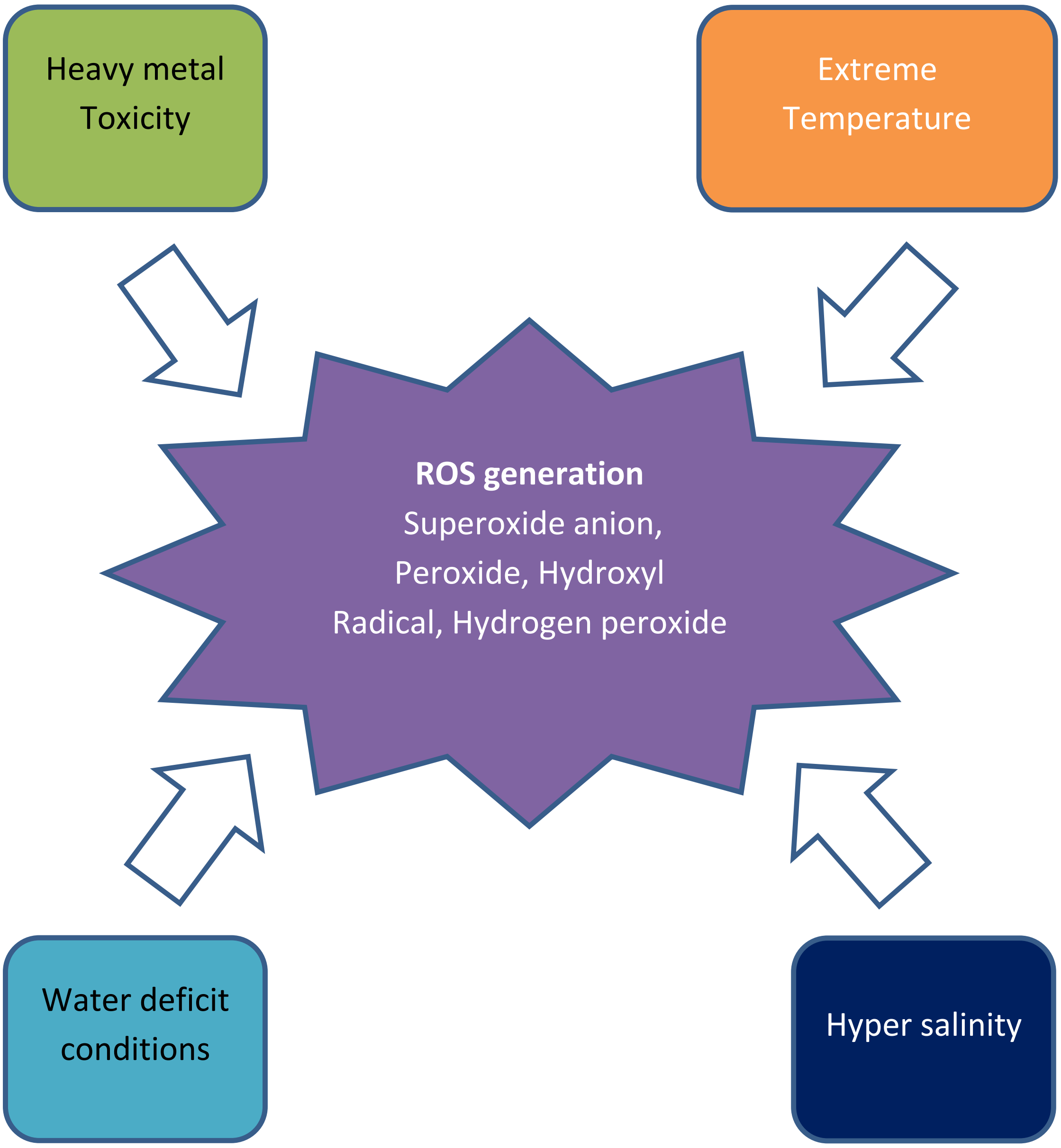

Fig 2.2: Abiotic stresses in crops

2.12 Oxidative stress in plants

Oxidative stress in plants occurred due to the disturbance in equilibrium between reactive oxygen species and antioxidant activity. Lack of antioxidant in plant cell leads to decrease scavenging of oxidative species, which give rise to excessive production of free radicals. These radicals are highly detrimental for plants, as they damage the molecular structure of proteins, lipids, and carbohydrates. Plants have developed certain antioxidative mechanism to scavenge free unstable oxygen rich species. Antioxidant components in plants comprises of two types of mechanisms. Enzymatic mechanism includes production of catalase, peroxidase, ascorbate peroxidase which catalyzes scavenging of harmful chemical species. Non-enzymatic antioxidative mechanisms disrupts free radical chain reaction process by producing carotenoids, glutathione, and phenol, etc. (Karuppanapandian et al. 2011).

Chapter 3

Methodology

This section describes the design of experimental approach that was adopted during this study. Experiments were carried out at Institute of Environmental science and Engineering (IESE), National Institute of Science and technology, Islamabad. In this study Silica particles and nano-silica particles were synthesized from rice husk using eco-friendly processes. Then wheat (*Triticum aestivum*) was grown in a lead contaminated soil containing 500 mg/kg, and the toxicity of wheat was dealt with common silica (300, 500 mg/kg) and nano-silica particles (300, 500 mg/kg). Keeping in view objectives of the study, the methodology of the study has been elaborated here in detail.

3.1 Preparation and characterization of nano- Silica particles

3.1.1 Preparation of Silica Nanoparticles

Rice husk was 1st heated in 10% weight of Hydrochloric acid solution for about 2 hours, then it was rinsed with de-ionized water for 5-10 minutes. After rinsing RH was dried in an oven at 100 °C for 24 hours. Dried rice RH was then pyrolyzed in a muffle furnace at 700 °C for two hours. After that it was ultrasonicated with 0.5M KNO_3 solution, followed by stirring. Then sample was filtered followed by drying for 4 hours at 100°C. Sample was then further pyrolyzed at 800°C for 8 hours (Wang et al. 2012) which yield pure nano-sized silica particles.

3.1.2 Characterization of nano-silica particles

3.1.2.1 X-ray diffraction (XRD)

X-ray diffraction (XRD) is a non-destructive technique, which is being used for the collecting accurate information regarding the physicochemical properties of materials. Composition, profiles amorphous, crystalline, and crystal lattice structures of materials are bring determined by XRD (Chauhan 2014). This analytical procedure was used to determine the phase of crystalline structure and planes of the material of nano-silica particles.

3.1.2.2 Scanning electron microscope (SEM)

Scanning electron microscope was invented after the discovery of Transmission electron microscope, but it took much longer time to develop into a practical tool for scientific research. Resolution power of SEM was improved after magnetic lens were added to it and with the addition of stigmator to the lens column. Application range of SEM was much broader now than TEM, SEM is quite extensively used in the field of medicine and semi-conductor industry (Shoukry 2011).

Scanning electron microscope is one of the supplements of optical microscope, and it can magnify an object 10X to 300000X with the resolution of few nanometers. Surface morphology of nano-silica particles were determined by SEM. Before scanning, the powdered silica nanoparticles were sonicated with 0.5M KNO_3 solution for 30 minutes.

3.2 Preparation and characterization of common silica particles

3.2.1 Preparation of Common silica particles

RH was first cleaned with tap water to remove dust and sand particles attached to it. After cleaning RH was dried in oven at 70° C for 24 hours. Dried RH was then carbonized in a muffle furnace at 400° C, carbonization was carried out to remove organic impurities from RH. Carbonization yields black colored material which undergo sintering in two phases. First it was sintered at 900°C for 2 hour producing RHA. In 2nd phase RHA was sintered at 800°C for 12 hours, this second phase of sintering remove all the metallic impurities present in RHA (Suryana et al. 2018). This RHA was rich in silica content, and it was characterized by XRD and SEM.

3.2.2 Characterization of common silica particles

3.2.2.1 X-ray Diffraction (XRD)

Phase of the crystalline structure, and planes of the common silica particles were analyzed by XRD. The XRD pattern of silica particles was attained using X-Ray Diffractometer (Theta-Theta STOE, Germany). n. Scan range of 20°-80° was used (2θ; λ = 0.154) was used with a step of 0.5° at 40 mA and 40 kV.

3.2.2.2 Scanning Electron Microscopy (SEM)

Silica nanoparticles’ surface morphology was obtained by SEM (JSM-6490A, JEOL) with a 20 kV accelerating voltage.

3.3 Soil preparation

Soil was taken from the premises of National University of science and technology (NUST), H-12 campus Islamabad. Soil was dried for two weeks by direct exposure of sun, to remove the moisture content. After drying, soil was grounded in Ball mill at Particulate Technology Laboratory, SCME, NUST, Islamabad, Pakistan.

3.4 Soil preliminary analysis and preparation for pot experiment

3.5 Soil characterization

Following characteristics of soil were analyzed soil pH, Electrical conductivity, Moisture content, water holding capacity, soil lead content, soil texture.

3.6 Soil pH

10g of air died sieved soil(<2mm) was taken in 100mL glass beaker. Then 50mL of distilled water added to 10g of soil. It was then left on a shaker for 30minutes at 180rpm. Then pH of the soil was determined using (HI 2211 pH Meter/ HANNA Instruments) (Lierop 2018).

3.7 Soil Electrical conductivity

10g of air died sieved soil (<2mm) was taken in 100mL glass beaker. Then 50mL of distilled water added to 10g of soil. It was then left on a shaker for 30 minutes at 180rpm. Soil EC value was then determined by putting EC meter electrode into the suspension, and value was taken, once reading gets stable (Meers et al. 2005).

3.8 Soil Organic matter content

Dry combustion method was used to evaluate organic matter content of soil. 10g of air-dried soil was taken in a China-dish, and it was heated in a muffle furnace at 350°C for 3 hours. After that China-dish was placed in a desiccator for 30 minutes. once it was cooled then it was reweighted to find the percentage of organic matter content of soil (Wang et al. 2015).

3.9 Soil moisture content

10g of soil was taken in a petri-dish, then it was oven-dried at 105°C overnight. It was then removed from oven and placed in a desiccator for 30 minutes, and then it was again weighted. Following relation was used to determine the moisture content of soil.

Soil moisture % = = Wet soil − Dry soil /Dry soil × 100

3.10 Soil texture

Saturation paste method was used for quantitative measurement of soil texture. 100g of air-dried soil was taken in 100mL container, then distilled water was added slowly and mixed uniformly, until a saturated paste was obtained. Saturated paste equals the weight of water which was required to saturate the 100g of soil sample. According to USDA soil classification, soil texture was calculated.

Table 3.1 USDA soil classification system based upon soil moisture contents

0-20	Sandy or loamy
20-35	Sandy loam
35-50	loam or silt loam
50-65	clayey loam
65-80	Clay
>81	organic soil

3.11 Soil water holding capacity

10g of air-dried soil was weighed, then Whatman filter paper no. 42 is placed on a funnel. Weighted soil was then placed on the filter paper and 10mL of water was poured on to the soil. Filtrate was collected in a graduated cylinder. Then final volume of filtrate was noted and from the filtrate water holding capacity of soil was computed.

3.12 Lead content in soil

Soil sample was first homogenized and then oven dried at 105°C. After that 1.0g of soil was taken and 3mL of HNO_3/H_2O_2 mixture (2/1) was added to the soil. Mixture was gently shaken and then dried on a hot plate. After cooling the sample 2 mL of nitric acid was added and centrifuged. Clear digest was then analyzed by AAS (Atomic absorption spectroscopy) (Bakirdere and Yaman 2008).

3.13 Preparation of pots

Pots were filled with 5 kg of soil, followed by proper labelling of pots. Lead in the form of $PbNO_3$(500 mg/kg) was added before two weeks of sowing. Then common silica (300 mg/kg, 500 mg/kg) and nano silica (300 mg/kg, 500 mg/kg) were added after 1 week of adding lead. 8 seeds of wheat were sown in each pot. After on one week of sowing only 5 uniform seedlings were kept in pots, others seedling was removed from pots, so that every seedling would grow efficiently with required space.

3.14 N, P, K fertilizers

After 20 days of sowing, pots were fertilized by 120 kg/hectare of Urea, 50 kg/hectare of diammonium phosphate and 25 kg/hectare of potassium sulphate as a source of nitrogen, phosphorous and potassium, respectively.

3.15 Assessment of Chlorophyll Content

Chlorophyll content of wheat leaves were measured on daily basis. Measurements were taken using portable chlorophyll absorbance meter (CCM-200 Plus). Chlorophyll meter weighs 168 g with a 0.73 cm^3 measurement area and calculates chlorophyll content index (CCI) based on the absorbance measurements.

3.16 Analysis of wheat

After 80 days of sowing, oxidative stress and antioxidant enzymatic assays of wheat were performed.

3.17 Determination of Electrolyte leakage and hydrogen peroxide content

Electrolyte leakage (EL) content of wheat leaves value were determined using the procedure of (Dionisio-Sese and Tobita 1998). 200mg fresh leaves of wheat were rinsed with distilled water, then leaves were cut into small segments of 1cm and placed in tubes containing 10mL of distilled water. These samples were then placed at 32°C for two hours and EC_1 of the solution was evaluated using EC meter, then samples were placed at the temperature of 121°C for 20 minutes, after cooling the solution EC_2 of the solution was determined and EL can be calculated using equation

$$EL = (EC_1/EC_2) \times 100$$

For the determination of hydrogen peroxide 0.5mL of supernatant was extracted from fresh wheat leaves (0.5g) in about 5mL of K-P buffer and then centrifuged at 10,000 rpm. This supernatant was then mixed with 5mL of Trichloroacetic acid (TCA) (0.1%W/V) and 1mL of 10 micro molar potassium iodide buffers having pH 7. Absorbance of the solution was determined at 390nm (Junglee et al. 2014).

3.18 Determination of Malondialdehyde MDA content

For the measurement of MDA content fresh wheat leaves (0.25g) were homogenized in 5 mL of 0.1% trichloroacetic solution (TCA), and mixture was then centrifuged at 3000g for about 10 minutes. After that 1.0 mL of supernatant was collected, and it was then used in 4mL of 0.5% Thiobarbituric acid at 100°C for about 30 minutes, again it was centrifuged and cooled to room temperature. Absorbance of the solution was evaluated at 532nm, and non-specific absorbance of the solution was calculated at 600nm, and this value was subtracted from the actual absorbance, then co-efficient was used to determine the value of MDA (Heath and Packer 1968).

3.19 Catalase determination

Catalase content in wheat leaves was determined by calculating the disappearance of hydrogen peroxide. 0.5mL of 75 mM hydrogen peroxide was added in 1.5 mL of phosphate buffer (pH=7), and then 50 micro liter of enzyme extract used in reaction mixture. Decrease in absorbance was observed for 1 minute and enzymatic activity was then calculated by measuring the amount of disappeared hydrogen peroxide (Sarker and Oba 2018).

3.20 Measurement and analysis

Before harvesting, plant height and spike lengths were determined with the help of stainless-steel meter rod, and number of spikes in each pot were counted.

3.21 Post-harvest analysis

Once wheat attained physiological maturity, it was harvested; roots, shoots, spikes, and husks were separated with the help of scissors. Aboveground biomass which includes shoots and grains were washed with de-ionized water. Roots were washed with tap water then with dilute acid and finally with de-ionized water (Khan et al. 2020).

3.22 Determination of dry weight

Washed biomass was then oven-dried at 70°C for 48 hours. Then root, shoot and husk dry weight was determined with weighing balance. Plant samples were then stored in labeled sampling bags.

3.23 Lead analysis in wheat

3.24 Wet digestion method

After harvesting and oven drying lead content in root, shoot and grain was determined by wet digestion method. 0.1g of plant samples (root, shoot, grain) were crushed. In a volumetric flask 0.1g of plant sample was added to concentrated nitric acid and hydrochloric acid (3:1) through pipette each time when process of wet digestion had taken place. After that flask was placed on a hot plate in fume-hood. On hot plate temperature was increased gradually from 50˚C to 150˚C. Heating continued till color of the mixture became transparent, and all the plant material was completely digested. Once solution became colorless, samples were taken off from hot plate and filtered via Whatman No.52 filter paper. In a volumetric flask filtered solution was diluted with distilled water to make the final volume of about 50mL, and then it was stored at 4˚C for Pb analysis (Saifullah et al. 2010). Lead was then determined by atomic absorption spectrophotometer (AAS).

Statistical Analysis

Data obtained from experimental work was subjected to statistical analysis by calculating means and standard deviation of each group. Multi-group analysis was performed using one-way analysis of variance. Means were compared using Tukey HSD test. $p<0.05$ was considered as statistically significant.

Chapter 4

Results and Discussion

4.1 Characterization of common silica particles

4.1.1 XRD Results of common silica particles

The crystalline size and phase composition of common silica particles were determined through XRD analysis as shown in Figure 4.1. Solid diffraction peaks between 20.52-22.7° indicates that material is of amorphous is nature. Absence of any other peak also suggests that these particles are amorphous in nature. Results are in coherence with finding of several other studies, diffraction peaks at 2-theta between 20-30° documented to the characteristic peak of silica.

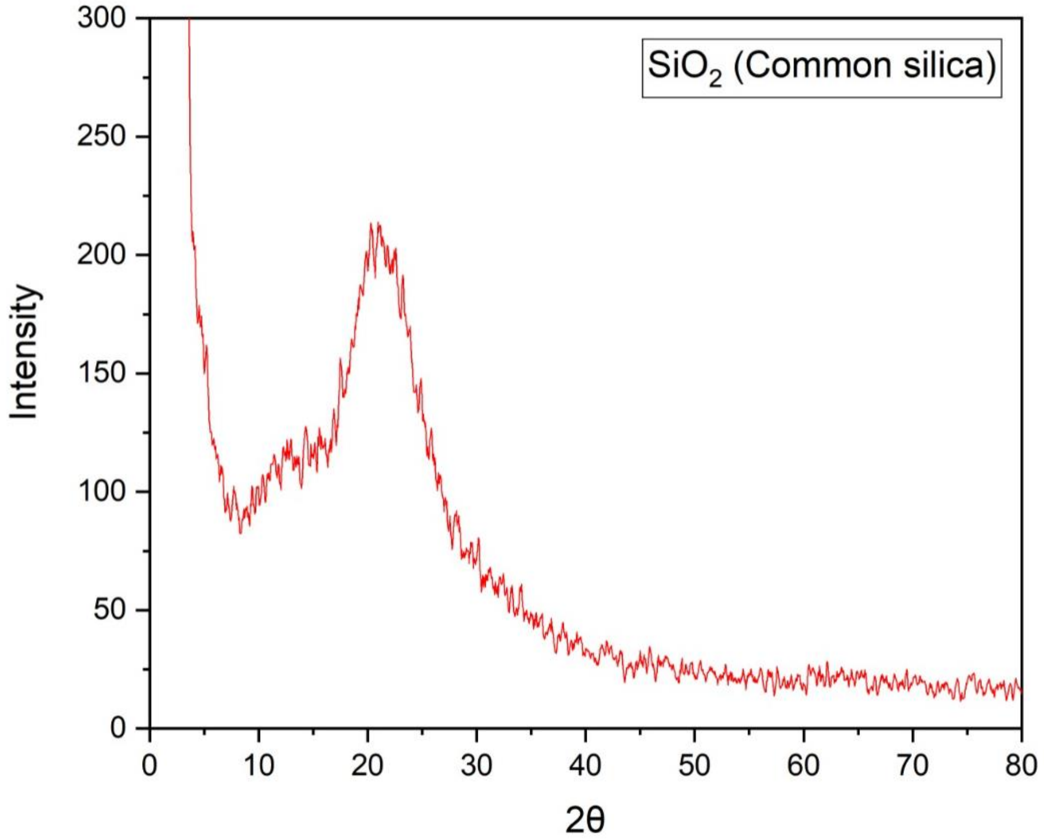

Fig 4.1: XRD results of common silica

4.1.2 SEM result of common silica particles

Surface morphology of Silica particles was estimated by SEM. The image at a magnification of 15K X shows pure Silica particles, (Fig 4.2) which are amorphous in nature.

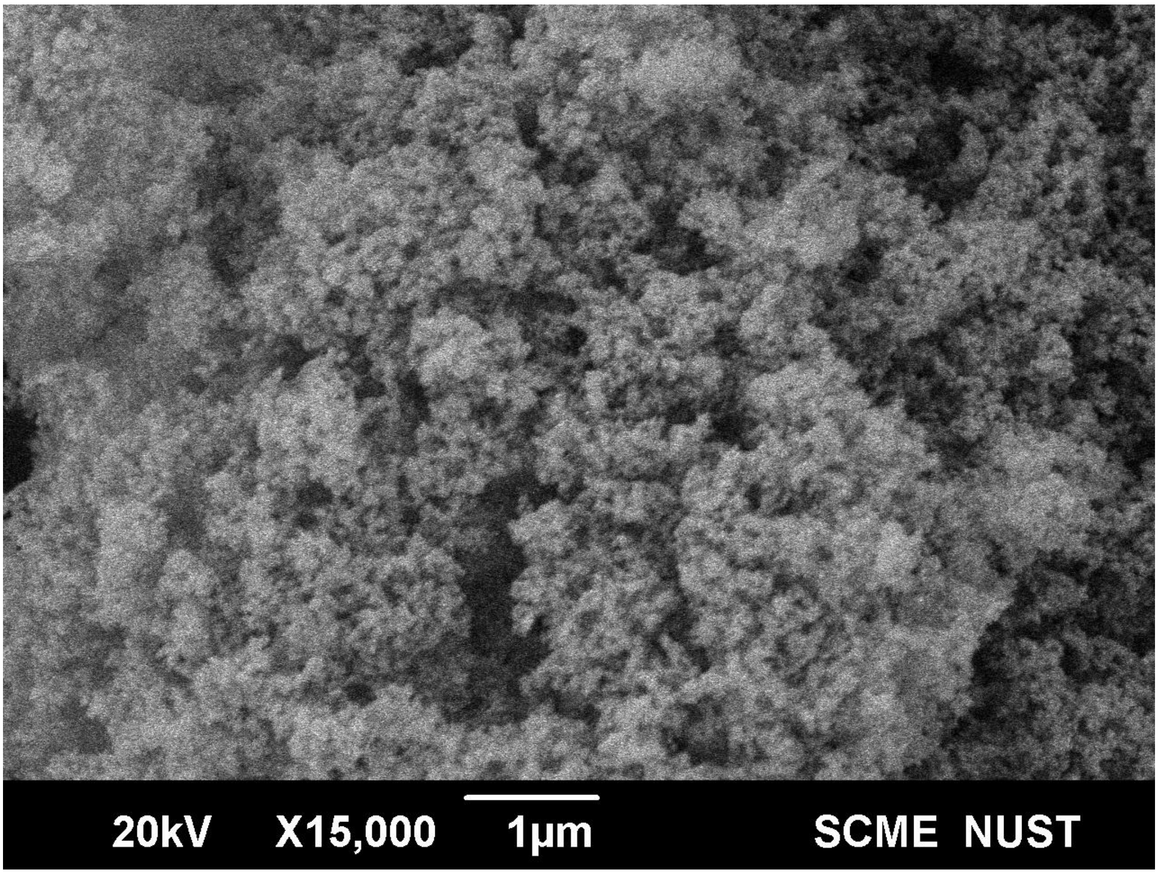

Fig 4.1: SEM image of common silica

4.2 Characterization of common silica particles

4.2.1 XRD result of nano-silica particles

The crystalline size and phase composition of the prepared nano silica particles were determined through XRD analysis as shown in figure 4.3. Solid diffraction peaks between 20.52-22.7° demonstrate presence of silica particles. Results are in coherence with finding several other studies diffraction peaks at 2-theta between 20-30° documented to the characteristic peak of silica.

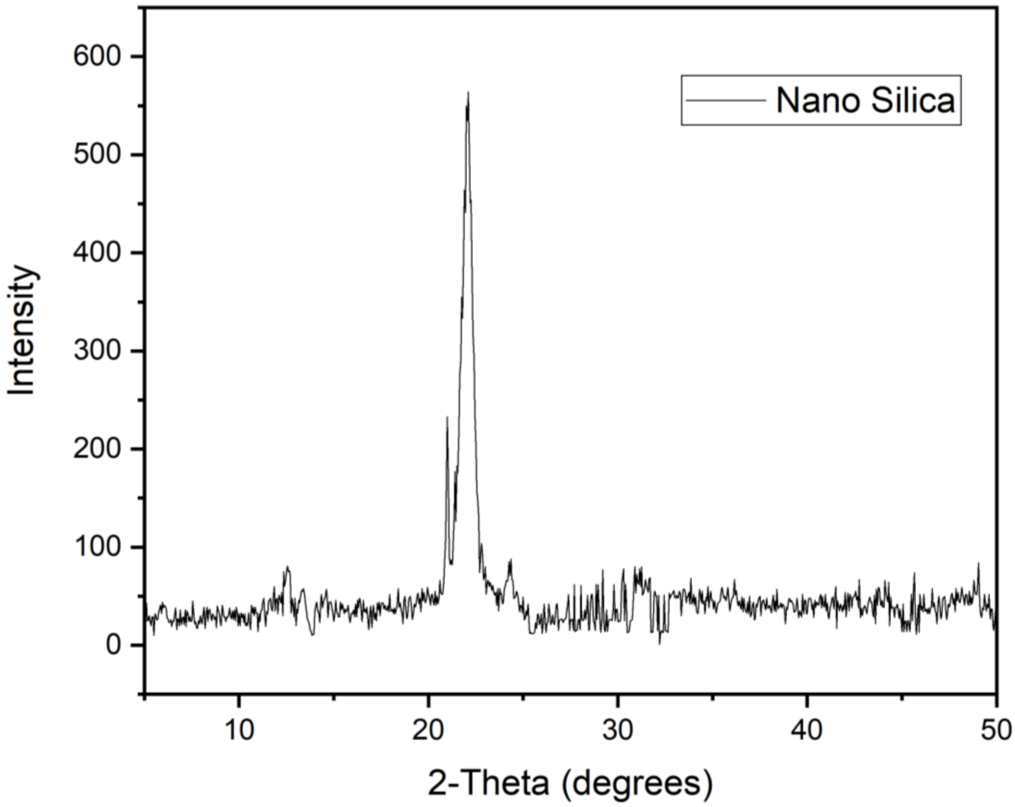

Fig 4.3: XRD results of nano-silica

4.2.2 SEM result of nano silica particles

Fig 4.4 shows the image of nano-silica particles by JEOL JSM-6640 at 30K magnification. Image confirms the presence of porous, sponge like structure of high roughness and complexity. Such surface indicates high surface area with estimated average size of about 90nm.

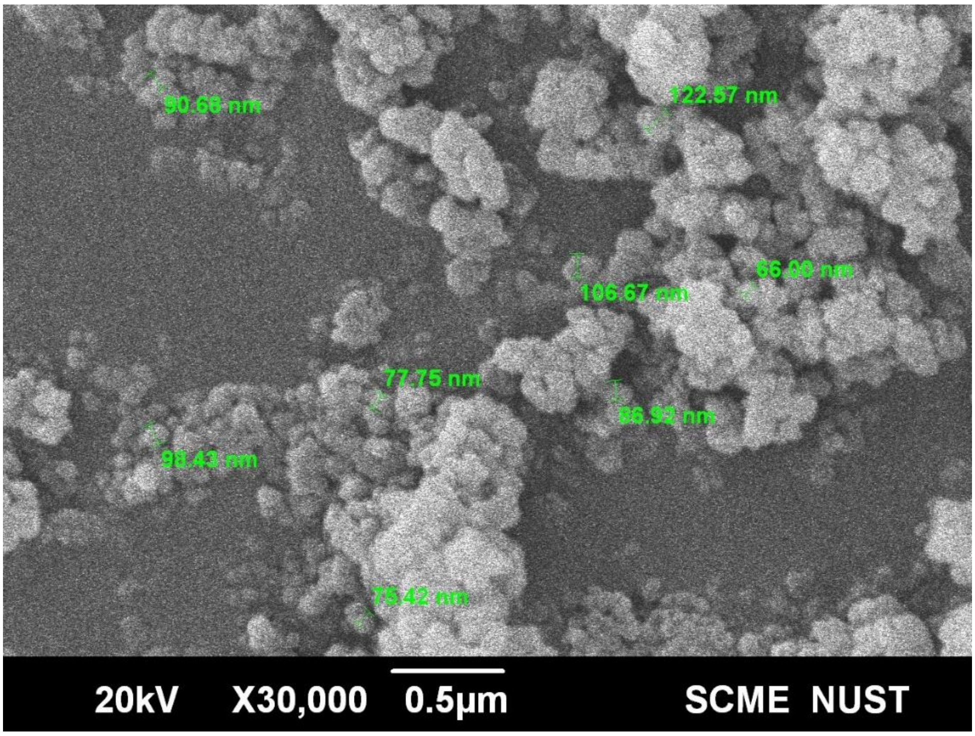

Fig 4.4: SEM image of nano-silica

4.3 Growth response of wheat to silica particles

4.4 Plant height

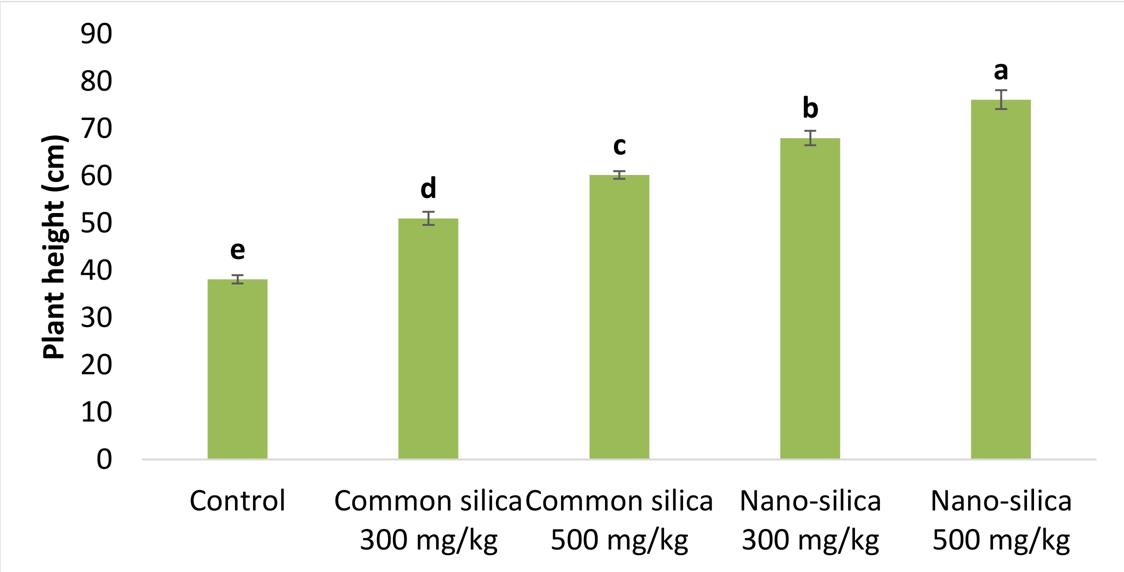

Fig 4.5: Plant height upon application of different levels of nano and common silica

Fig 4.5 is showing the impact of common silica and nano-silica particles on wheat height under lead stress. Highest plant height was observed when plant was treated with highest quantity of silica nanoparticles, while lowest value was observed in control group where silica was absent. Results revealed that maximum mean plant height (76 cm) was measured in plants grown in 500 mg kg^{-1} of silica nanoparticle while lowest mean value (38.1 cm) was observed in pots where silica was absent. Various studies have shown that silica increases plant height and improves physiology. Silicon is deposited in the cell wall of plants, hence strengthening the leaves and stem, hence increasing height.

Silica particles were able to enhance the wheat root, shoot weight which ultimately increases plant height (Walsh et al. 2018). Silicon nanoparticles positively impacted wheat growth in wheat in Cd contaminated soil, highest values of plant height was observed which wheat was treated with maximum quantity of silica nano particles, while lowest height was observed in control where no silica was applied (Khan et al. 2020).

4.5 Shoot dry weight

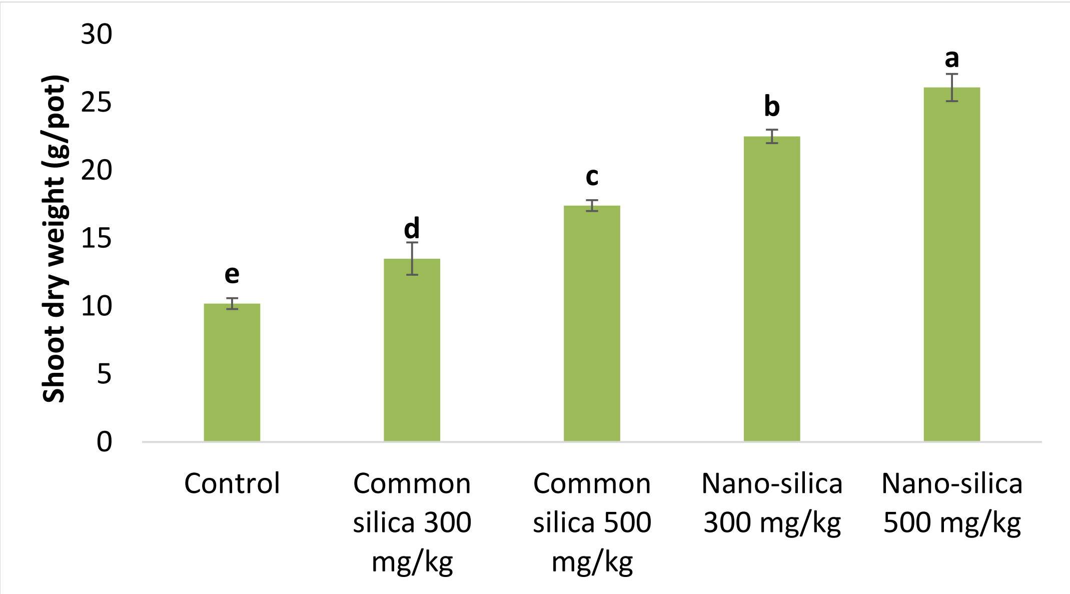

Fig 4.6: Shoot dry weight of wheat upon application of different levels of nano and common silica

Impact of common silica and nano-silica particles on shoot dry weight under lead stress is shown in fig 4.6. Wheat shoot weight was maximum at 500 mg kg^{-1} dose of silica nanoparticle. Results indicated that the maximum shoot weight (26.2 g/pot) was recorded when wheat was treated with 500mg/kg of silica nanoparticles, while lowest shoot weight (12.3 g/pot) was observed in control group where no silica was applied

Lead toxicity decreased shoot dry weight of wheat (Mehboob et al. 2018) while shoot dry weight of wheat under cadmium toxicity increased when it was treated with silica nano-particles (Khan et al. 2020). Shoot dry matter greatly increased when wheat is treated with 150mg/kg of silicon compared to 50 mg/kg of silicon under water deficit conditions (Ahmad et al. 2007).

4.6 Root dry weight

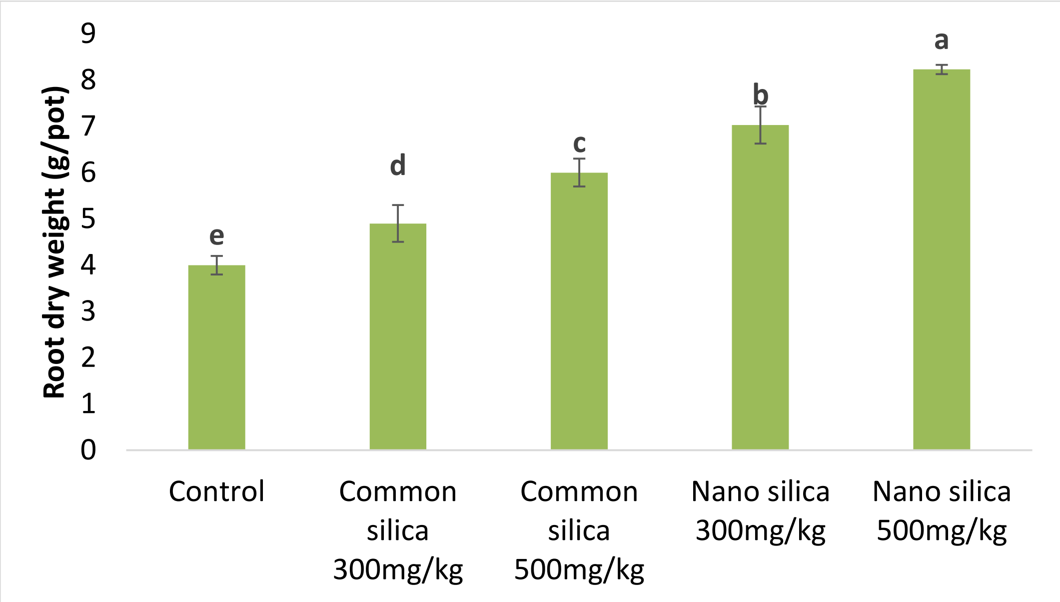

Fig 4.7: Root dry weight of wheat upon application of different levels of nano and common silica

Fig 4.7 is showing the root dry weight of wheat under lead toxicity when it was treated with different doses of common silica and nano-silica particles. The highest mean value for root dry weight (8.2345 g/pot) was found in wheat treated with 500 mg/kg of silica nanoparticles, while minimum (4.00 g/pot) was found in control containing no silica.

Lead toxicity in wheat inhabit root activity and decreases its weight (Mehboob et al. 2018) while silica nano-particles when apply in greater quantity significantly increased root dry weight of wheat under heavy metal stress (Ali et al. 2019).

It was reported (Guo et al. 2006) that silicon application promote root growth, increased the number of secondary roots in *Medicago sativa*. Silicon effects were more noticeable in root than in shoot.

4.7 Spike length

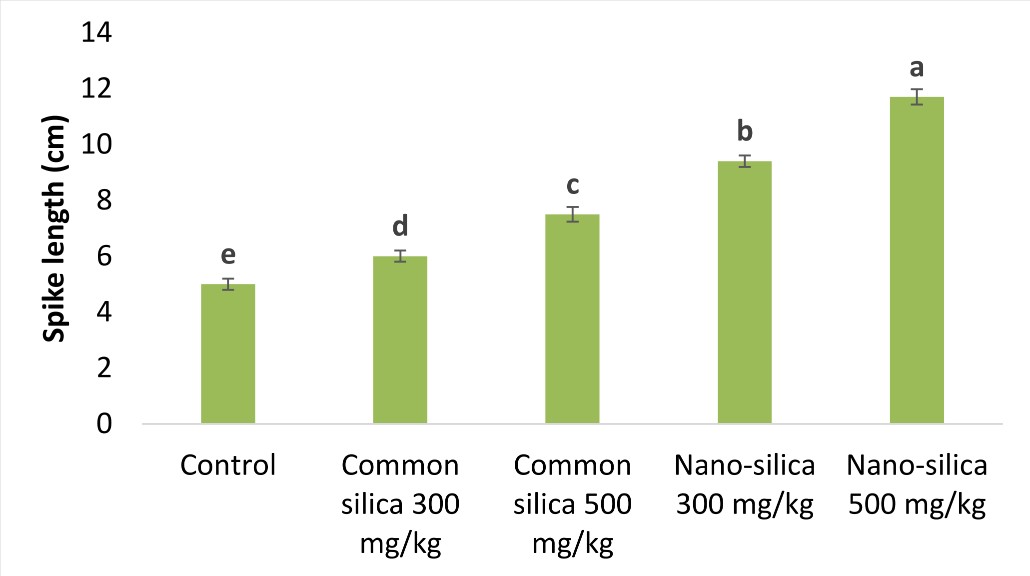

Fig 4.8: Spike length of wheat upon application of different levels of nano and common silica

Fig 4.8 is showing spike length of wheat under lead stress when treated with different doses of common silica and nano-silica particles. The highest wheat spike length (9.4 cm) was observed when it was treated with highest quantity of nano-silica particles, while minimum spike length (5 cm) was noticed in control where silica was absent.

Lead toxicity in wheat inhabits shoot growth, due to the accumulation of lead in shoot area which results in the reduction of meristematic cells. Lead toxicity in wheat induces enzyme disruption which greatly compromises growth (Sharma et al. 2018). Heavy metal toxicity in wheat reduced spike length in wheat, but silicon nanoparticles are able to enhance spike length under heavy metal toxicity (Ali et al. 2019).

(Rizwan et al. 2019) reported that **c**admium toxicity reduced spike length of wheat, while zinc oxide and iron oxide nanoparticles increased spike length under cadmium toxicity. Nanoparticles promote the uptake of essential nutrients in wheat, which provides strength to the plant shoot and increases the size of spike.

4.8 No. of spikes per pot

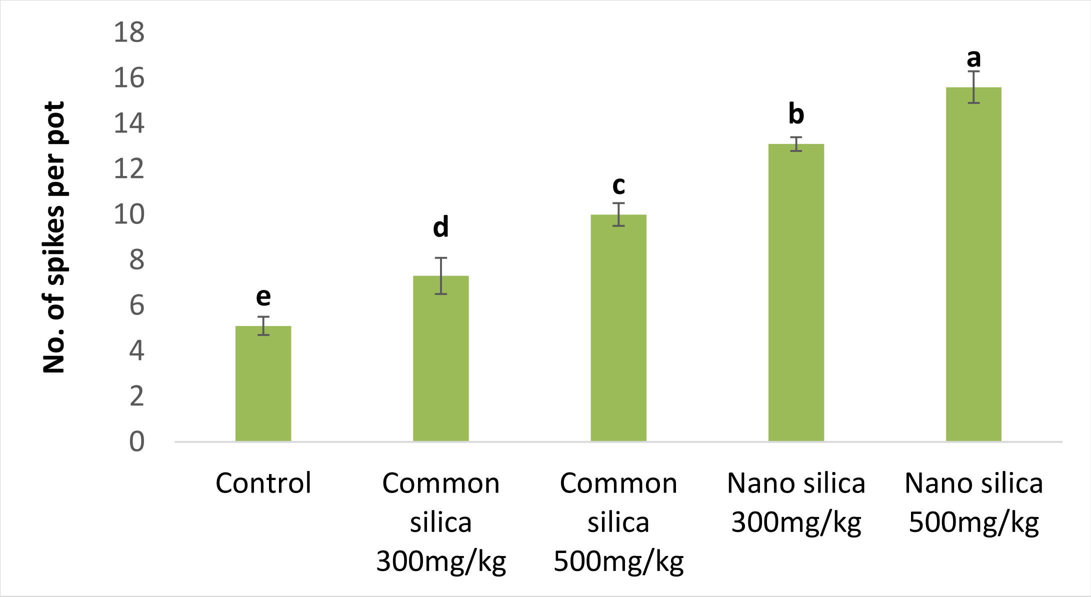

Fig 4.9: No. of spikes per pot of wheat upon application of different levels of nano and common silica

Fig 4.9 is showing number of spikes which were able to grow in each pot. Maximum number of spikes (5) were recorded in pots containing 500mg/kg of nano-silica particles, while lowest number of spikes (14) were found in control where no silica was applied. Marked improvement in physiological parameters of wheat were observed with silica application, including number of spikes. Number of spikes in wheat decreased significantly under heavy metal stress, but when silicon nano-particles were applied it promotes spike length and also increased number of spike in each pot (Khan et al. 2020) .

Number of spikes in each pot decreased significantly under cadmium toxicity and water deficit conditions, but silicon nanoparticles amendments increased the number of spikes in each pot (Khan et al. 2020).

4.9 Husk dry weight

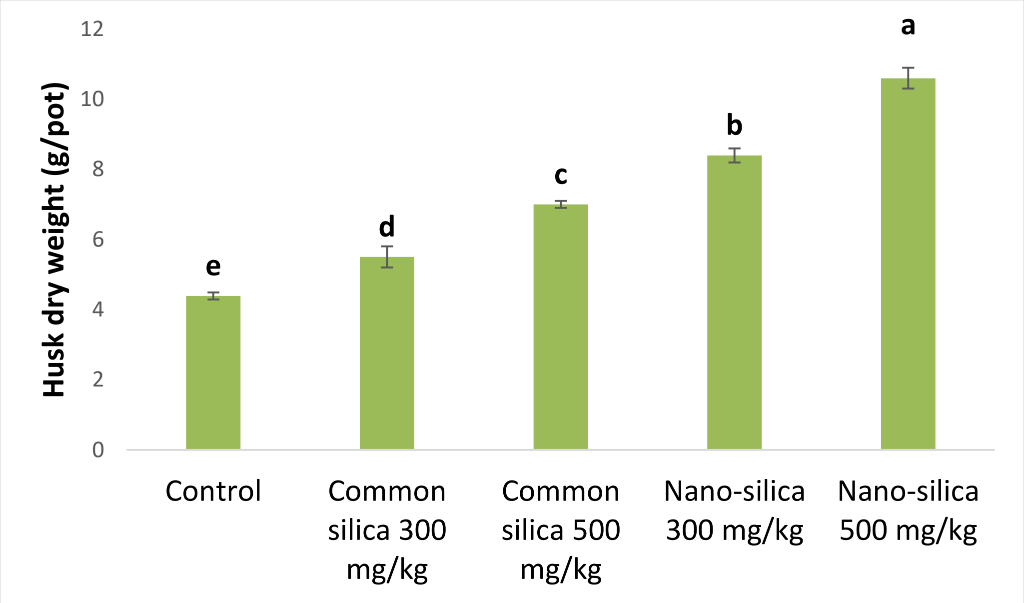

Fig 4.10: Husk dry weight of wheat upon application of different levels of nano and common silica

Fig 4.10 is showing husk dry weight of wheat under lead stress when treated with different doses of common and nano-silica particles. Husk dry weight was found to be maximum (5 g/pot) when wheat was treated with maximum quantity of nano-silica treatments, while lowest value (9.6 g/pot) was reported when no silica was applied. Husk dry weight decreased significantly under heavy metal stress(Rizwan et al. 2019) while silica particles of nano-size are able to counter the negative effect of heavy metal stress by increasing husk growth and its weight (Ali et al. 2019). Husk weight in wheat is negatively affected by lead toxicity, while ascorbic acid amendment tackle negative impact of lead toxicity by enhancing husk dry weight (Alamri et al. 2018).

4.10 Grain weight

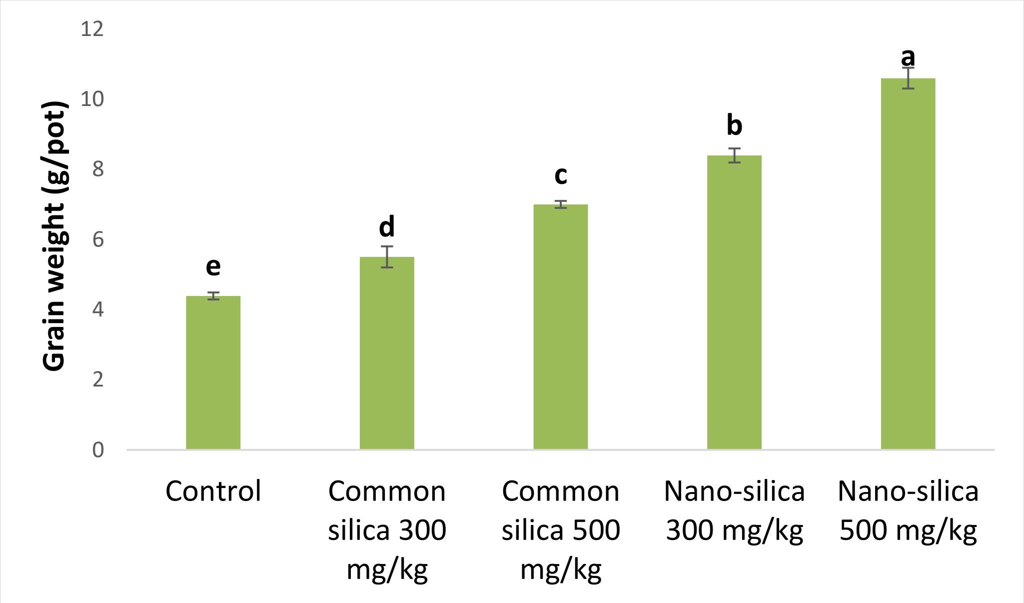

Fig 4.11 Wheat Grain weight upon application of different levels of nano and common silica

Impact of common silica and nano-silica particles on wheat grain weight under lead stress is shown in fig 4.11. Grain weight was maximum at 500 mg kg^{-1} dose of silica nanoparticle. Results indicated that the maximum grain weight (8.6 g/pot) was recorded when wheat was treated with 500mg/kg of silica nanoparticles, while lowest grain weight (4.00 g/pot) was observed in control group where no silica was applied. Lead toxicity in wheat decrease grain weight due to reduced uptake of essential nutrients from soil (Kanwal et al. 2020). . At soil amendment of 900 mg/kg Si NPs grains dry weights per pot were increased by 57%, as compared control(Ali et al. 2019). Wheat productivity greatly increased by silicon application, as it enables the crop to remain erect by maintaining water status. This erectness of leaves accounts for 10% increase in photosynthetic activity (Alamri et al. 2018).

4.11 Chlorophyll content

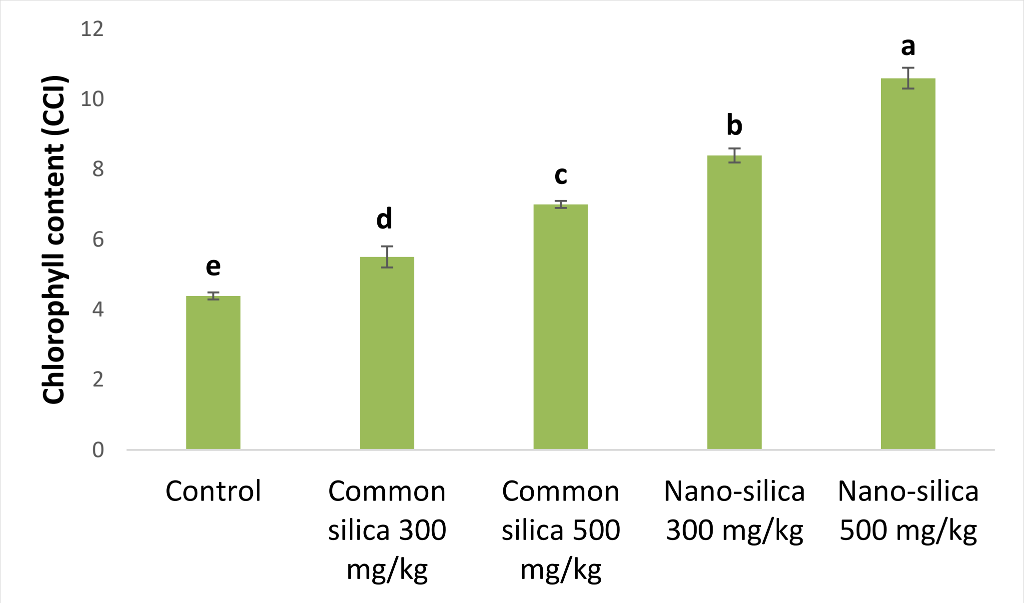

Fig 4.12: Chlorophyll content of wheat leaves upon application of different levels of nano and common silica

Fig 4.12 is showing chlorophyll content of wheat under lead stress when treated with different doses of common and nano-silica particles. Chlorophyll content was maximum (4.42) when wheat was treated with 500mg/kg nano-silica treatments, while lowest value (10.02) was reported when no silica was applied.

Lead toxicity decreased chlorophyll in wheat, by causing oxidative stress in wheat (Lamhamdi et al. 2011). Similar results were reported by (Alamri et al. 2018) as wheat subjected to lead stress has the highest value of chlorophyll degradation, while it has lowest value for total chlorophyll content.

Chlorophyll contents increased significantly with silica nanoparticles. Chlorophyll content was highest when maximum quantity of nano-particles (100 mg/kg) were applied in a heavy metal contaminated soil.(Ali et al. 2019).

Silicon application enhanced chlorophyll a as well as chlorophyll b concentration as compared to the control (where silicon wasn't applied) in salt stressed sweet pepper plants (Abdelaal et al. 2020).

4.12 Malondialdehyde

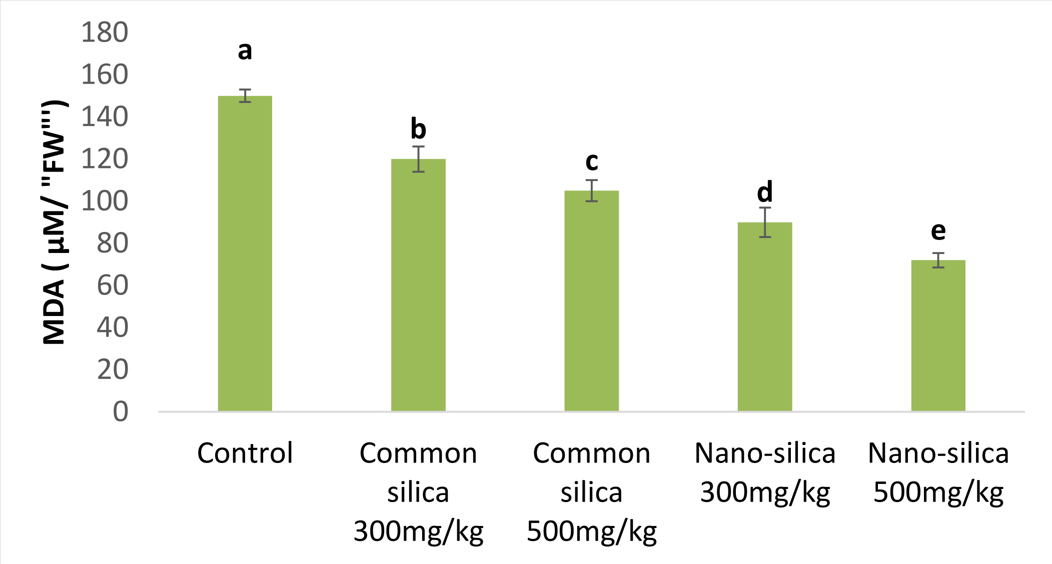

Fig 4.13: MDA content of wheat leaves upon application of different levels of nano and common silica

Fig 4.13 is showing values of malondialdehyde content of wheat under lead stress when treated with different doses of common and nano-silica particles. MDA was the lowest (4.06 µM/g) when wheat was treated 500mg/kg of nano-silica, while its value is maximum in control group, where no silica was applied. (Alamri et al. 2018) reported that MDA attained maximum value when wheat was grown in lead containing medium. lipid peroxidation (MDA content) was decreased significantly by applied silicon under excess boron toxicity (Gunes et al. 2007).

MDA product of free radical mediated lipid peroxidation is a marker of lipid oxidative injury. Highest value of MDA was noticed in heavy metal stressed wheat, while its value decreased under soluble silica. Application of soluble silica increases number of protons which were able to neutralize negative hydroxyl group, hence countering the negative impact of lipid peroxidation.

4.13 Electrolyte leakage

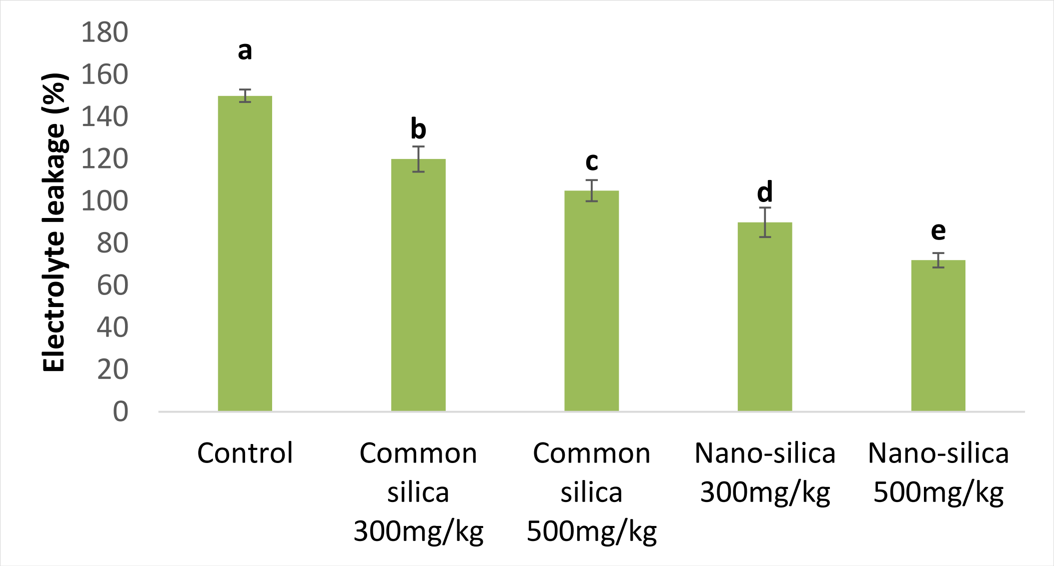

Fig 4.14: Electrolyte leakage of wheat leaves upon application of different levels of nano and common silica

Impact of common silica and nano-silica particles on wheat electrolyte leakage (EL) under lead stress is shown in fig 4.14. EL content was minimum (82%) when wheat was treated with 500mg/kg nano-silica treatments, while maximum value (46%) of EL was reported when no silica was applied. EL content increased significantly under cadmium toxicity, but its value was decreased by nano silicon application because silicon maintain cell membrane integrity, limiting the loss of electrolyte from leaf (Ali et al. 2019).

Application of calcium silicate is quite effective in decreasing electrolyte leakage content in salt stressed wheat. Electrolyte leakage value was significantly higher in the solution where silicon was absent (Ali et al. 2012). The lowest values of EL were observed when wheat was grown under non-stressed conditions. When wheat was grown under lead containing medium values of EL jumped up, however, this value decreased significantly when wheat was treated with ascorbic acid amendment (Alamri et al. 2018).

4.14 Catalase

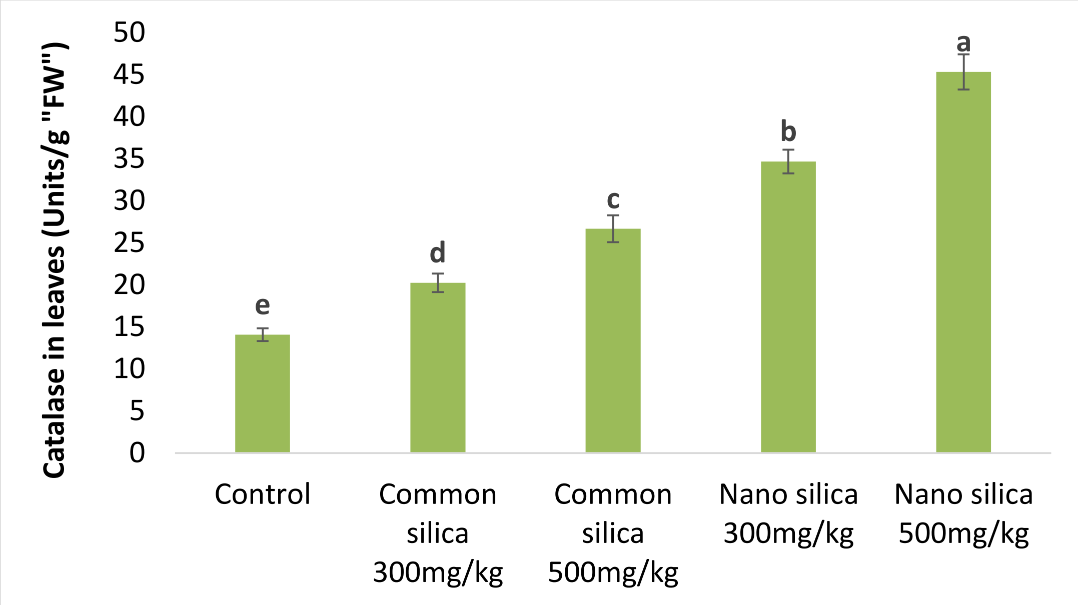

Fig 4.15: Catalase content of wheat upon application of different levels of nano and common silica

Impact of common silica and nano-silica particles on wheat catalase activity under lead stress is shown in fig 4.15. Catalase was highest (45.03) when wheat was treated with 500mg/kg nano-silica treatments, while lowest value (14.033) was obtained when no silica was applied.

Catalase activity in wheat decreased significantly when it was treated with lead (Alamri et al. 2018). Silicon application greatly increased catalase activity in *Solanum lycopersicum* under water stress condition (Shi et al. 2016). Saline stress significantly decrease catalase activity in wheat, but silicon amendment can activate antioxidative mechanism, by increasing catalase activity. This increase was more prominent as the experiment proceeds, this shows that catalase impact was time dependent, catalase content in leaves increase gradually with time (Liang et al. 2003).

4.15 Hydrogen peroxide

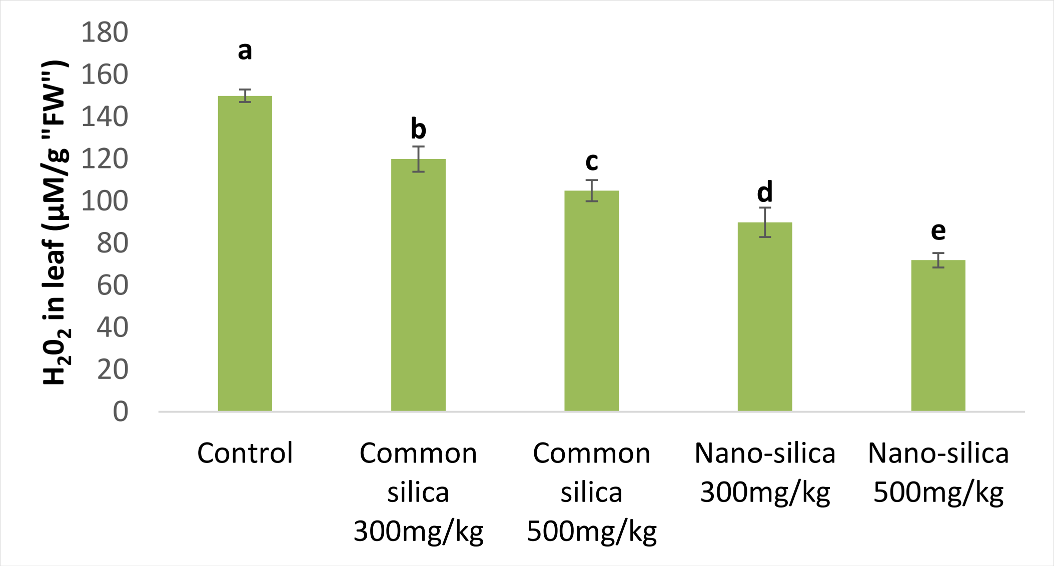

Fig 4.16: Hydrogen peroxide content of wheat upon application of different levels of nano and common silica

Fig 4.16 is showing hydrogen peroxide content of wheat under lead stress when treated with different doses of common and nano-silica particles. Hydrogen peroxide content was minimum (80 µM/g) when wheat was treated with 500mg/kg nano-silica treatments, while its value is maximum (160 µM/g) in control group where no silica was applied. Lead induced oxidative stress in wheat by raising hydrogen peroxide content (Alamri et al. 2018) while silicon counter toxic impact of heavy metal toxicity by lowering hydrogen peroxide content (Khan et al. 2020).

Hydrogen peroxide also decreased significantly under silicon treatment in salt stressed sweet pepper plant, as silicon has potential to counter saline stress by scavenging hydrogen peroxide like species (Abdelaal et al. 2020).

Concentration of hydrogen peroxide was significantly higher in lead stressed wheat. Excessive concentration of hydrogen peroxide causes oxidative damage in wheat by inducing a Haber-Weiss reaction. Ascorbic acid amendment counters the negative impact of lead stress by encouraging hydrogen peroxide scavenging, and ultimately lowering its concentration (Alamri et al. 2018).

4.16 Pb concentration of wheat root

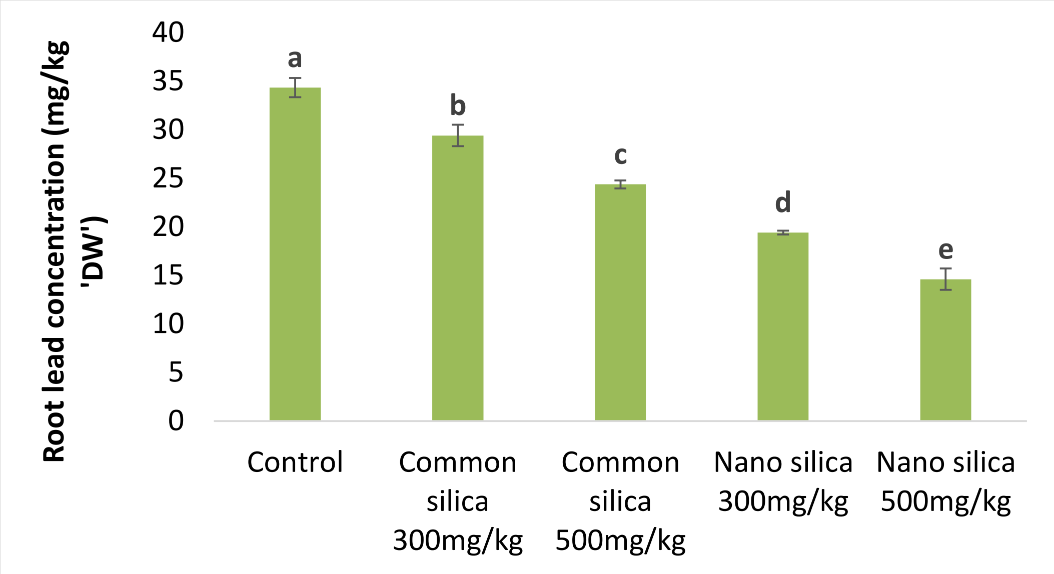

Fig 4.17: Root Pb concentration upon application of different levels of nano and common silica

Fig 4.17 describes quantity of lead enters from soil to wheat root in the presence of common silica and nano-silica under different doses. Maximum quantity of lead can enter root when no silica was applied, common silica was able to restrict lead entry. However, minimum quantity of lead enters wheat root when (500mg/kg) silica nanoparticles were applied.

Silica particles tend to immobilize lead within soil, reducing bioavailability of lead thus restricting movement of lead. Similar results were reported by (Ali et al. 2019) that silica particles tent to limit cadmium entry from soil to wheat roots, while common silica and nano-silica application was able to counter lead toxicity in rice by preventing its entry into roots (Liu et al. 2015). Significant amount of lead enters from soil to wheat root, when no treatment was applied, highlighting adverse impact of lead on root. But Diammonium phosphate treatment greatly decreases concentration of lead entering root. This reduction can be attributed to the increase in pH, and immobilization of Pb within soil, restricting its entry into root (Rehman et al. 2017).

4.17 Pb concentration of wheat shoot

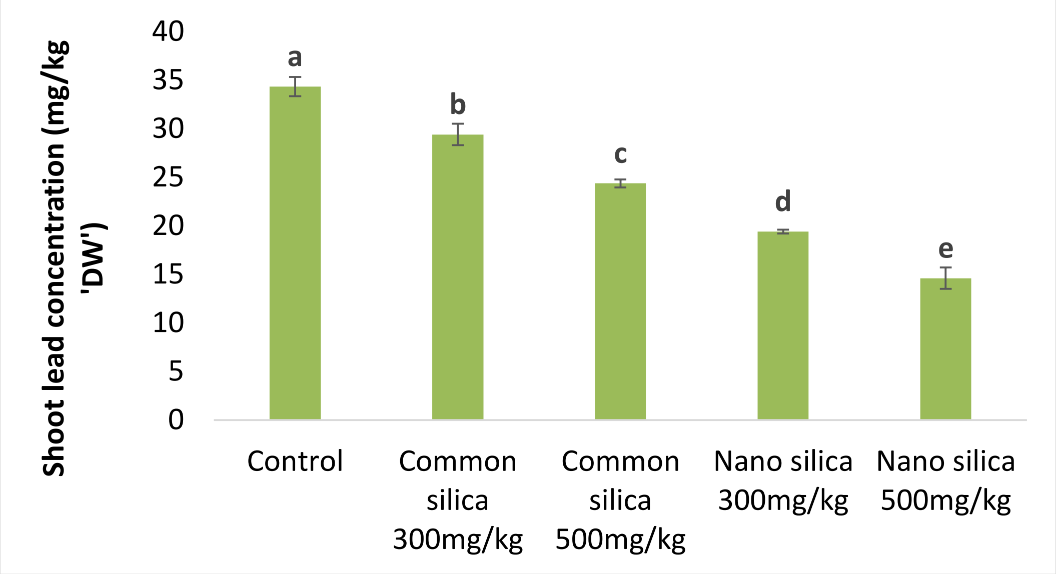

Fig 4.18: Shoot Pb concentration upon application of different levels of nano and common silica

Fig 4.18 describes translocation factor of lead from root to shoot in the presence of common silica and nano-silica under different doses. Translocation factor decreased when common silica particles were applied, but reduction was more pronounced when nano-silica particles were applied, as only (17.33 mg/kg) lead enters shoot when nano silica was used in comparison to (34.33 mg/kg) in case of control group where no silica was applied.

Silica application greatly reduced translocation factor of lead from rice root to shoot, but this effect was more significant when nano particles were applied (Liu et al. 2015). Translocation factor (from root to shoot) of cadmium decreased by 16.9%, 35% and 46.8% when silica nanoparticles were applied in 25, 50 and 100 mg/kg quantity compared to control group where no silica particles were applied (Khan et al. 2020).

4.18 Lead concentration of wheat grain

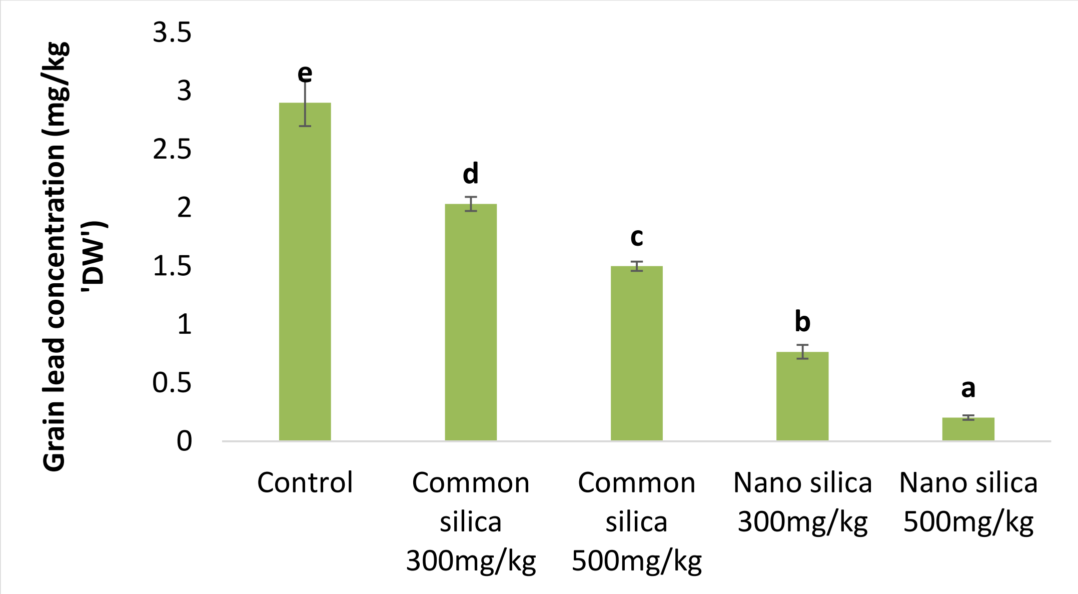

Fig 4.19: Grain Pb concentration upon application of different levels of nano and common silica

Fig 4.19 describes translocation factor of lead from shoot to grain in wheat when different doses of common silica and nano-silica were applied. Translocation factor of lead decreased when common silica particles were applied, but reduction was more pronounced when nano-silica particles were applied, as only (17.33 mg/kg) lead enters shoot when nano silica was used in comparison to (34.33 mg/kg) in case of control group where no silica was applied. Translocation factor (from shoot to grain) of cadmium decreased 15.3%, 25%, and 35.2% in 25, 50, and 100 mg/kg NPs, respectively, from the control where silica wasn't applied(Khan et al. 2020).

Translocation of lead from shoot to grain also decreased in rice under common silica and nano-silica treatments. When soil was treated with two different concentrations of lead (500, 1000 mg/kg), reduction in translocation factor of lead ranged from 8.3-13.7% under common silica treatment, while it varies from 15.3%-21.1% in case of nano-silica treatment (Liu et al. 2015). Translocation factor of cadmium significantly decreased under surface modified nano-silica application. Cadmium quantity decreased by 47.95% when surface modified nano-silica was applied in comparison to a control where no amendment was added (Wang et al. 2020).

Table 4.1: Analysis of control and spiked soils

	Post-harvest soil analysis					
Soil characteristics	Initial soil analysis	Control group	Common silica 300mg/kg	Common silica 500mg/kg	Nano-silica 300mg/kg	Nano-silica 500mg/kg
pH	7.47	6.9	8.1	8.2	7.9	8.1
Electrical conductivity mS/m	6	5.8	6.21	6.27	6.49	6.71
Moisture content %	12	10	12	13	12	15
Organic matter content %	0.35	0.3	0.45	0.55	0.46	0.61

Table 4.1 shows the impact of lead, common silica, and nano-silica on certain physico-chemical characteristics of soil. This table shows the impact of common silica and nano-silica on soil pH, Electrical conductivity, organic matter, and soil moisture content. Column highlighted with yellow color shows initial parameters of soil. Control group describes changes in soil parameters which happens because of lead contamination in soil. Other column denotes the impact of common silica and nano silica of varying concentration on soil. Soil pH decreased with addition of lead, but it increased when silica is added. Soil organic matter content, moisture content and organic matter content also showed similar trends, that lead application decreased their value, but silica tends to enhance these parameters. Increase in soil pH could be attributed to the fact that silica increase soil reaction and help in correcting soil acidity by neutralizing exchangeable Fe, Al and Mn and other toxic elements. Increase in electrical conductivity might be attributed to submergence, increase in solubility of salts present in the soil and due to the dissolution of silicon fertilizers. Increase in soil organic matter content was due to the reason that silica application increased mineralization rate, which in turn increases the soil organic matter content. Soil moisture content also increased under silica application, due to strong swelling capacity of silica which increased soil porosity which increased soil moisture content (Das et al. 2021).

Chapter 5

Conclusions and Recommendations

5.1 Conclusions

High purity common silica and nano-silica particles were obtained from rice husk using eco-friendly processes. Lead toxicity caused oxidative stress in wheat altering various physiological and biochemical processes. Root, shoot, husk, and grain dry weight decreased under lead toxicity, while hydrogen peroxide content, electrolyte leakage and malondialdehyde increased under lead stress in wheat. Silica applications enhanced wheat tolerance to Pb toxicity, by increasing root weight, shoot weight, husk weight and grain weight. Silica particles counter the toxicity of lead by increasing catalase, chlorophyll content and plant height and by decreasing hydrogen peroxide content, malondialdehyde and electrolyte leakage content of wheat leaves. Silicon application decreased translocation factor of lead from roots to shoots and from shoot to grain. The observed effects were greater for nano-Si treatment than that for common Si treatment, as nano-silica particles are more effective in countering the negative impacts of lead than normal sized particles.

5.2 Recommendations

Several useful recommendations can be derived from the current study, few of them are mentioned here:

Further research would be required under diverse geographical conditions by growing various crops to ascertain the potential of silica in tackling heavy metal toxicity.

- Silica is considered green alternative to conventional technologies used to tackle heavy metal stress in plants. Further optimization studies are required in different soil types and various plant species.
- Nano-fertilizers must be prioritized in agriculture sector because they are more efficient in enhancing the productivity of crops. For this purpose, detailed studies are required for standardization of size, phase, application mode and agronomic practices required for the application.
- Agricultural residues may have differential silica yield capacities. Detailed studies are required to explore best quality and yield from various sources.

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