



Foliar use of TiO₂-nanoparticles for okra (*Abelmoschus esculentus* L. Moench) cultivation on sewage sludge-amended soils: biochemical response and heavy metal accumulation

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Abstract

Considering its richness in organic and inorganic mineral nutrients, the recycling of sewage sludge (SS) is highly considered as a soil supplement in agriculture. However, the fate of hazardous heavy metal accumulation in the crops cultivated in SS amended soils is always a source of concern. Since nanoparticles are widely recognized to reduce heavy metal uptake by crop plants; thus, the present experiment deals with okra (*Abelmoschus esculentus* L. Moench) cultivation under the combined application of SS and TiO₂-nanoparticles (NPs). Triplicated pot experiments were conducted using different doses of SS and TiO₂-NPs such as 0 g/kg SS (control), 50 g/kg SS, 50 g/kg SS + TiO₂, 100 g/kg SS, and 100 g/kg SS + TiO₂, respectively. The findings of this study indicated that among the doses of treatment combinations investigated, 100 g/kg SS + TiO₂ showed a significant ($p < 0.05$) increase in the okra plant yield (287.87 ± 4.06 g/plant) and other biochemical parameters such as fruit length (13.97 ± 0.54 cm), plant height (75.05 ± 3.18 cm), superoxide dismutase (SOD: 110.68 ± 3.11 μ/mg), catalase (CAT: 81.32 ± 3.52 μ/mg), and chlorophyll content (3.12 ± 0.05 mg/g fwt.). Also, the maximum contents of six heavy metals in the soil and cultivated okra plant tissues (fruit, stem, and root regions) followed the order of Fe > Mn > Cu > Zn > Cr > Cd using the same treatment. Bioaccumulation and health risk assessment indicated that foliar application of TiO₂-NPs significantly reduced the fate of heavy metal accumulation under higher doses of SS application. Therefore, the findings of this study suggested that the combined use of SS and TiO₂-NPs may be useful in ameliorating the negative consequences of heavy metal accumulation in cultivated okra crops.

Keywords Health risk assessment · Heavy metals · Nanomaterials · Sewage sludge · Target hazard quotient

Introduction

The drastic increase in the human population has resulted in uncontrolled settlements, leading to the generation of a huge volume of domestic and municipal wastewaters (Malik et al. 2015; Hube and Wu 2021). These wastewaters contain several harmful pollutants which are responsible for the degradation of soil–water compartments and their associated

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living biota (Sabeen et al. 2018). It is estimated that out of the total domestic wastewater produced in 2020, only 56% was sufficiently treated and disposed of which suggests that progress is still challenging around the globe (UNWW 2021). Moreover, the problem remains worst in most developing countries due to the lack of efficient wastewater treatment technologies (Mara 2013; Sato et al. 2013; Adelodun et al. 2021).

Sewage sludge (SS) is a semi-solid substance produced from sedimentation, aeration, coagulation, and other steps of domestic wastewater treatment plants (Kumar et al. 2022). SS comprises residual soil particles, organic and inorganic compounds, pesticides, heavy metals, pathogens, etc. making it rich in various pollutants (Qian et al. 2016). The management of SS is also crucial as it can easily contaminate different environmental components. However, prior to its disposal, SS is being widely utilized for various purposes such as anaerobic biogas generation, incineration, pyrolysis, wet-air oxidation, biochar, and biofertilizer production (Cieřlik et al. 2015).

Sewage sludge, being rich in various organic and inorganic nutrients, is widely accepted as a fertilization source for arable soils (Eid et al. 2019). In the sewage treatment plants, the produced sludge is digested to harness biogas and then air-dried to obtain dewatered fertilizer (Cieřlik et al. 2015). It is reported that the barren soils nourished with SS could be rehabilitated for better agricultural use which also minimizes the chemical fertilizer usages (Li et al. 2009). SS has been successfully utilized for the cultivation of various crops including wheat (*Triticum aestivum*; Eid et al. 2019), broad bean (*Faba sativa*; Eid et al. 2018), soybean (*Glycine max*; Rodrigues Prates et al. 2020), maize (*Zea mays*; da Silva et al. 2021), and rice (*Oryza sativa*; Zuo et al. 2021), among many others.

The successive use of SS depends on several factors such as the availability of nutrients, toxicants, and aftereffect-intensity on the soil (Tytla 2019). However, the SS utilization in arable soil fertilization comes with some drawbacks. These include excessive mineral nutrients, heavy metal accumulation, pathogen's introduction, the killing of beneficial microbial communities, and drastic changes in soil structure and pH (Tagg et al. 2021). The introduction of certain pollutants may increase their bioavailability in the plants, which may lead to stress and rapid bioaccumulation in the plant (Goala et al. 2021; Eid et al. 2021a).

Some highly toxic pollutants such as poly-aromatic-hydrocarbons (PAHs), pesticide residues, heavy metals, and other carcinogens disturb the plant-root physiology and nutrient uptake patterns, leading to oxidative stress in plants and consequently non-feasible crop yields (Lin et al. 2021). Several heavy metals, including cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), arsenic (As), and lead (Pb), are abundantly found in SS, which create adverse effects on plant growth, crop yields,

and also health risks to the consumer (Yakameran et al. 2021). Therefore, the problem relating to SS fertilization, i.e., heavy metal accumulation, needs to be adequately investigated and addressed.

Recent advances in material sciences have contributed to developing efficient formulations for agricultural uses such as nano-pesticides, nano-formulations, and nano-fertilizers, among many others (Jampilek and Kráľová 2015). In this, nanoparticles (NPs) are chemical substances having particle sizes between 1 and 100 nm. NPs can easily release the desired substances locally into plant cells to stimulate their growth and productivity (Pestovsky and Martínez-Antonio 2017). Nowadays, various types of NPs are widely available in the cultivation of different crops due to their various potential benefits in alleviating the plant's ability to absorb harmful chemical species, including heavy metals (Panpatte et al. 2016; Zhou et al. 2021). A report by Ogunkunle et al. (2020) showed that foliar application of TiO₂-NPs resulted in decreased uptake of Cd in the cowpea plant. Especially, TiO₂-NPs are considered one of the most effective nano-formulations, which can reduce heavy metal bioaccumulation in various crops (Irshad et al. 2021). Foliar use of TiO₂-NPs at low concentrations can easily support the plant leaf/root system against the chemical stress caused by heavy metal pollutants and inhibit their migration to upper parts by accelerating the biochemical defense mechanisms (Hong et al. 2021). It has been reported that TiO₂ application can aid the agro-business due to its several properties such as it improves plant productivity and quality by boosting the activities of the enzyme, increasing photosynthetic pigments, facilitating nutrient utilization by root systems, suppressing the uptake of harmful elements, mediating stress tolerance, and increasing overall crop yield (Chaudhary and Singh 2020).

Meanwhile, SS in this study, as polluted fertilizer, may bring the heavy metals into soils and thereby uptake by the experimental plant. Therefore, considering the above problems, the present study is designed to alleviate the risk of heavy metal uptake by the okra (*Abelmoschus esculentus*) plant under the combined use of dewatered SS and TiO₂-NPs. Additionally, the health risk of heavy metal accumulation is investigated in order to ensure the edibility of the cultivated okra fruit.

Materials and methods

Collection of experimental materials

For the present study, SS was collected from 27 million liters per day (MLD) capacity sewage treatment plant (Jagjeetpur, Haridwar, India) (29°54'01.2"N 78°08'14.9"E), while the garden soil was collected from Gurukula Kangri

(Deemed to be University), Haridwar, India (29°55'10.3"N 78°07'08.3"E). Sterile polyethylene bags (5 kg capacity) were used to collect both SS and soil samples. Besides this, the healthy and fresh seeds of the okra (*Abelmoschus esculentus*) hybrid variety (ANMOL) were procured from Seeways Agriseeds Pvt. Ltd. (New Delhi, India). It is widely grown in India and is considered one of the most pest-resistant varieties (Ali et al. 2021). Moreover, laboratory-grade TiO_2 -NPs were also procured from Akshar Agro Chemicals (Junagarh, India) for the foliar fertilization of okra. TiO_2 ($\text{O}=\text{Ti}=\text{O}$) is an odorless, solid white compound having a molecular mass of 79.866 g/mol, and a density of 4.23 g/cm³ (Chen and Selloni 2014). All materials were stored under laboratory conditions until further use in the crop cultivation experiments.

Experimental setup, treatment design, and cultivation conditions

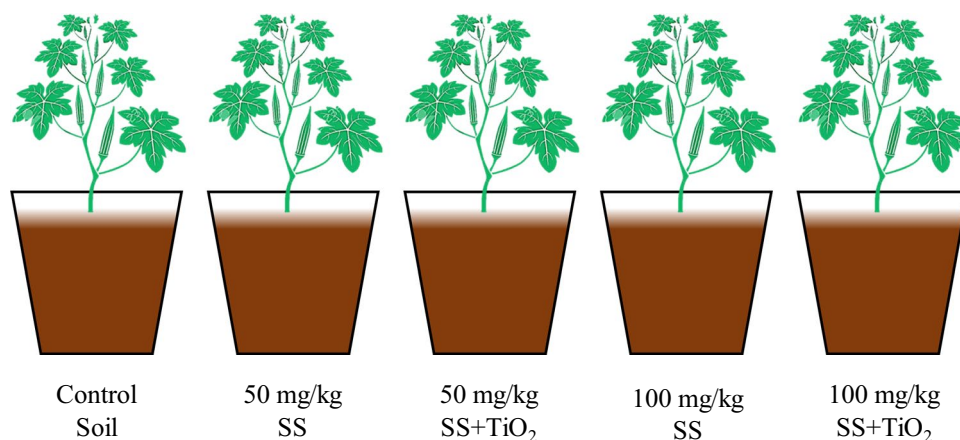
The laboratory experiment was carried out inside the greenhouse of the Department of Zoology and Environmental Science, Gurukula Kangri (Deemed to be University), Haridwar, India using 10 kg capacity round polyvinyl chloride (PVC) pots, with three replications of each trial. The pots were loaded with a mixture of garden soil and SS. A total of five experimental treatments including 0 g/kg (T1: garden soil as control), 50 g/kg (T2: 950 g soil + 50 g SS), 50 g/kg (T3: 950 g soil + 50 g SS + TiO_2 -NPs), 100 g/kg (T4: 900 g soil + 100 g SS), and 100 g/kg (T5: 900 g soil + 100 g SS + TiO_2 -NPs), respectively (Fig. 1). The design of the experiments was a completely randomized block design. The soil of the loamy class collected from the departmental garden was used in the present experiment. Both soil and SS were air-dried and sieved through a mesh size of 2 mm before implementing in the experiment. All pots were sown with a total of three okra seeds on March 18, 2021 and equally watered using a potable supply. The average temperature, humidity, and light intensity of the greenhouse were

24 °C, 74%, and 6000 lx (16/8 light/dark regime), respectively. The seedlings were allowed to grow for 15 days until the foliar TiO_2 -NP application in the T3 and T5 treatments. For this, a suspension was made by dissolving 2.5 mg of TiO_2 -NPs (average particle size of 25 nm) in 1000 mL distilled water and placing it in an ultrasonic water bath for 30 min. A calibrated hand sprayer (500 mL) was used for foliar application on the 15th, 30th, and 45th days of the okra cultivation experiment. The upper plant parts (leaves and stem) of okra plants were carefully cleaned using a low-pressure vacuum cleaner to remove any adhering dust/soil particles before NP application. Okra cultivation experiment was carried out for 90 days, and the total yield obtained in the first fruit harvesting (60th day) was represented as effective crop yield (g/plant).

Physicochemical, heavy metals, and instrumental analyses

In the present study, the collected soil and SS samples were pooled and analyzed for selected physicochemical and heavy metal parameters. For this, pH and electrical conductivity (EC: dS/m) were analyzed using a microprocessor-based digital pH-EC meter (Model-1611, ESICO, IN). Organic matter (OM: mg/kg), total Kjeldahl's nitrogen (TKN: mg/kg), and total phosphorus (P: mg/kg) were determined by titrimetric (Walkley and Black, 1934), acid digestion-distillation, and spectrophotometric (Cary 60 UV-Vis, Agilent, USA) methods, respectively (AOAC 2005; Kumar et al. 2020b, 2021). The contents of heavy metals in soil, sludge, and plant samples were subjected to di-acid (2:1; HNO_3 - HClO_4) digestion and further analyzed using an inductively coupled plasma atomic emission spectroscopy (ICP-OES: 7300 DV, Perkin Elmer) instrument (Eid et al. 2018). Moreover, the procured TiO_2 -NPs were subjected to Fourier's transform infrared spectroscopy (FTIR-8400S, Shimadzu, USA).

Fig. 1 Experimental design and setup of the pots for okra cultivation



Biochemical response analysis of okra plants

The effect of TiO₂-NP application on the biochemical response of okra cultivated in SS fertilized soil was evaluated in terms of superoxide dismutase (SOD), catalase (CAT), and total chlorophyll content (ChlP) activities. The SOD and CAT enzyme activities were estimated at 430 and 240 nm wavelength (Cary 60, UV–Vis, Agilent, USA) while using K-phosphate buffer and sodium hypochlorite extraction solutions to prepare the extraction solution. For chlorophyll estimation, a total of 1 g okra plant leaf sample was thoroughly crushed into 5 mL of 80% acetone solution. The contents were adjusted to 50 mL and homogenized using a high-speed centrifuge instrument (10,000 rpm). The residue was discarded, and the supernatant was used for ChlP estimation as described by Kumar et al. (2020a).

Data analysis and statistics

The uptake of heavy metals by the okra plant was enumerated using the bioaccumulation factor (BAF) index (Pachura et al. 2018). BAF was calculated based on the ratio of heavy metal concentration (mg/kg) in okra plant (HM_p) tissue and SS amended soil (HM_s) (Eq. 1). The form of formula is given below:

$$\text{Bioaccumulation factor} = \text{HM}_p / \text{HM}_s \quad (1)$$

On the other hand, the health risk assessment of individual heavy metal accumulation by okra plant was assessed using the target hazard quotient (THQ) index as given below (Eq. 2) (Ametepey et al. 2018; Kumar et al. 2021):

$$\text{THQ} = 10^{-3} \times (EE \times ET \times CR \times C) / (BW \times AT \times RfD) \quad (2)$$

where *EE* is the heavy metal exposure efficiency (365 days/year), *ET* is the exposure timespan (70 years), *CR* is the consumption rate (2.2 g/day), *C* indicates the concentration of heavy metals (mg/kg) in okra plant tissue, *BW* is the average body weight of the vegetable consumer (70 kg for adult and 16 kg for child), and *AT* is the average consumption time in days (25,550 days). Meanwhile, *RfD* indicates the reference dose of heavy metals viz., 5.0×10^{-4} , 4.2×10^{-2} , 3.0×10^{-3} , 7.0×10^{-1} , 1.4×10^{-2} , and 3.0×10^{-1} mg/kg/day for Cd, Cu, Cr, Fe, Mn, and Zn, respectively (USEPA 2011).

Finally, the health risk index (HRI) of okra consumption was calculated as a sum of THQs of all heavy metals as given in the equation (Eq. 3):

$$\text{HRI} = \sum \text{THQ}(\text{Cd} + \text{Cr} + \text{Cu} + \text{Fe} + \text{Mn} + \text{Zn}) \quad (3)$$

Moreover, Pearson's correlation matrix analysis was done in order to study the effect of different soil parameters and TiO₂-NPs on the biochemical and heavy metal accumulation response of okra. Data were analyzed using Microsoft Excel 2019 (Microsoft Corp., USA) and OriginPro Student Version (2021b) (Origin Inc., MX) software packages. The significance level of the statistical tests was $p < F$ value of 0.05, i.e., 95% confidence interval.

Results and discussion

Characteristics of soil, sewage sludge, and TiO₂-NPs

In this study, the soil and SS samples were analyzed in advance for various physicochemical and heavy metal properties (Table 1). Results indicated that the SS obtained from STP, Jagjeetpur, Haridwar, India, had relatively higher nutrient characteristics as compared to

Table 1 Characteristics of the soil and sewage sludge used for okra cultivation experiments

Parameter	Control (garden soil)	Sewage sludge (SS)	Treatments	
			50 g/kg SS	100 g/kg SS
pH	7.40 ± 0.05	8.43 ± 0.03*	7.62 ± 0.04*	7.81 ± 0.05*
EC (dS/cm)	1.96 ± 0.03	6.55 ± 0.06*	2.45 ± 0.19*	3.10 ± 0.04*
OM (%)	1.05 ± 0.02	28.52 ± 1.540*	1.98 ± 0.07*	2.69 ± 0.08*
TKN (g/kg)	1.29 ± 0.03	33.52 ± 0.817*	2.56 ± 0.05*	3.19 ± 0.15*
P (g/kg)	1.93 ± 0.08	11.06 ± 0.29*	2.30 ± 0.10*	2.57 ± 0.06*
Cd (mg/kg)	0.150 ± 0.006	2.558 ± 0.012*	0.760 ± 0.023*	0.901 ± 0.006*
Cr (mg/kg)	2.700 ± 0.010	9.801 ± 0.035*	3.520 ± 0.017*	4.032 ± 0.024*
Cu (mg/kg)	6.550 ± 0.028	74.150 ± 2.403*	12.067 ± 0.048*	19.044 ± 0.152*
Fe (mg/kg)	18.238 ± 0.125	37.094 ± 1.110*	23.120 ± 1.302*	26.109 ± 1.004*
Mn (mg/kg)	5.117 ± 0.087	13.009 ± 0.121*	7.517 ± 0.061*	9.083 ± 0.082*
Zn (mg/kg)	3.024 ± 0.011	112.082 ± 3.860*	32.050 ± 2.540*	57.108 ± 3.976*

Values are mean ± standard deviation of three replicates

*Significantly different with garden soil as control group at $p < 0.05$

the garden soil. In particular, the selected physicochemical and heavy metal parameters were significantly higher ($p < 0.05$) in SS and its two subsequent treatment groups, i.e., 50 g/kg SS and 100 g/kg SS. As the dose of SS was increased from 0 to 100 g/kg, the soil nutrient parameters were also augmented vise-versa. On the other hand, the FTIR spectrum analysis of TiO_2 -NPs (agriculture grade) showed the presence of different peaks ($1/\text{cm}$) such as 590 (vibration of Ti-O-O), 1630 (amides), 1770 (asymmetrical C=O), and 3430 (N-H stretching) associated with their respective bonding groups (Fig. 2). Previously, SS has been widely analyzed for the presence of different nutrients. Recent studies by Shaltout et al. (2021) and Eid et al. (2021b) showed that the dried SS contained significant levels of various beneficial nutrient elements with some heavy metals, which can be sustainably utilized in the cultivation of different crops such as great millet and cabbage. Irshad et al. (2021) pointed out some important peaks as major features of chemically synthesized TiO_2 -NPs.

Effects of sewage sludge and TiO_2 -NPs on growth and biochemical attributes

Results of one-way ANOVA given in Table 2 showed that supplementation of SS and TiO_2 -NPs significantly ($p < 0.05$) increased the fruit length (cm), yield (g/plant), and plant height of the okra plant. Similarly, the biochemical parameters such as SOD (μmg), CAT (μmg), and ChlP (mg/g fwt.) were also accelerated under nutrient stress caused by SS application. However, the garden soil treatment itself was not productive to support the good growth and productivity of the okra plant. Overall, the maximum fruit length, yield, and plant height of okra were observed as 13.97 ± 0.54 cm, 287.87 ± 4.06 g/plant, and 75.05 ± 3.18 cm, respectively, using the combined application of 100 g/kg SS and TiO_2 -NPs. In addition, there was a significant increase in the growth and biochemical parameters of okra under the combined application of SS and TiO_2 -NPs compared to individual SS treatment. In this, SOD and CAT activities were highest as 110.68 ± 3.11 and 81.32 ± 3.52 μmg , respectively, along with maximum production of ChlP (3.12 ± 0.05 mg/g).

Fig. 2 FTIR spectrum of TiO_2 -NPs used for foliar application on cultivated okra plants

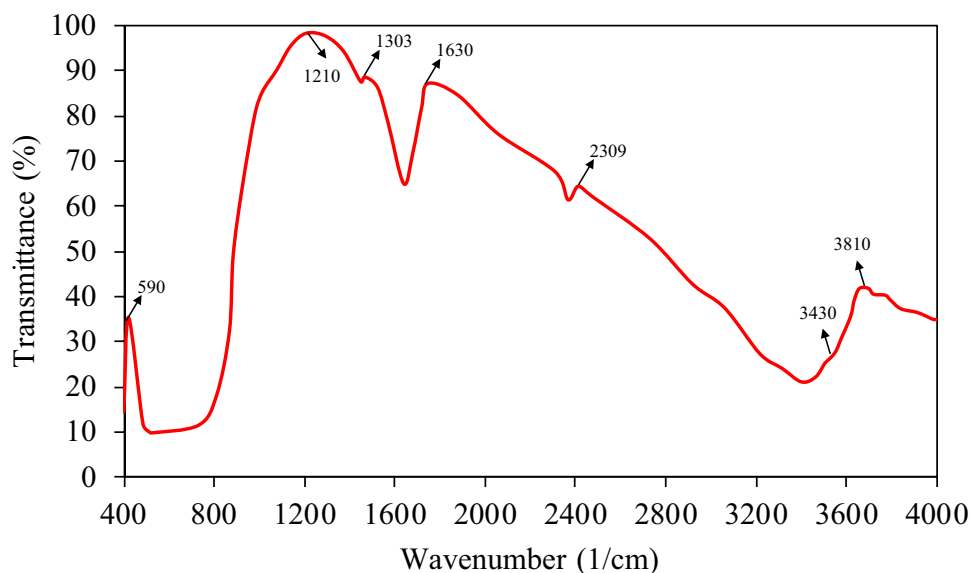


Table 2 Growth, yield, and biochemical parameters of cultivated okra as affected by sewage sludge and TiO_2 application

Treatment	Fruit length (cm)	Yield (g/plant)	Plant height (cm)	SOD (μmg)	CAT (μmg)	ChlP (mg/g fwt.)
Control	9.50 ± 0.35	168.45 ± 3.05	43.20 ± 2.50	78.08 ± 1.10	27.14 ± 3.28	2.06 ± 0.02
50 g/kg SS	$11.08 \pm 0.20^*$	$201.92 \pm 2.87^*$	$52.08 \pm 3.04^*$	$82.09 \pm 2.81^*$	$40.65 \pm 2.90^*$	$2.54 \pm 0.06^*$
50 g/kg SS + TiO_2	$12.13 \pm 0.45^*$	$236.09 \pm 5.22^*$	$60.40 \pm 4.10^*$	$89.15 \pm 3.44^*$	$53.08 \pm 4.62^*$	$2.78 \pm 0.04^*$
100 g/kg SS	$12.65 \pm 1.20^*$	$265.40 \pm 7.15^*$	$63.10 \pm 4.60^*$	$93.29 \pm 6.58^*$	$60.05 \pm 5.03^*$	$2.67 \pm 0.08^*$
100 g/kg SS + TiO_2	$13.97 \pm 0.54^*$	$287.87 \pm 4.06^*$	$75.05 \pm 3.18^*$	$110.68 \pm 3.11^*$	$81.32 \pm 3.52^*$	$3.12 \pm 0.05^*$

Values are mean $\pm$ standard deviation of three replicates

SS sewage sludge, SOD superoxide dismutase, CAT catalase, ChlP total chlorophyll content

*Significantly different with garden soil as control group at $p < 0.05$

The better growth and productivity of okra might be due to nutrient supplementation as a result of SS mixing. Since SS is considered a highly nutritive supplement for arable soils, it is commonly used to enhance crop production in India. Slightly improved growth of okra plant under combined use of SS and TiO₂-NPs might be due to plant-NP interaction, which alters the plant physiology. Correlation analysis also revealed that SS dose and TiO₂-NP application showed positive relations with the heavy metal characteristics of soil, yield, and biochemical attributes of the okra plant (Fig. 3).

The critical knowledge of nutrient delivery through nanoparticle-assisted formulation suggests that plant growth is strongly impacted by the size of the NPs, which allows them to enter the cell system (Rico et al. 2011; Ullah et al. 2020). Though, interaction may result in either positive or negative effects based on the type of NPs, their particle size, and applied dose. Some of the positive impacts are

an increase in light absorption, stress enzyme production, root and shoot growth, stomatal conductance, photosynthetic activities, fruit yield, and so on, whereas negative impacts include disturbance in nutrient-water acquisition system, genome damage, cellular structure damage, production of toxic metabolites, oxidation/reduction of beneficial biomolecules, etc. (Mittal et al. 2020; Shahid et al. 2020; Kumar et al. 2022). In our study, the foliar application of TiO₂-NPs did not show any negative impact on okra plant growth.

Previously, Ullah et al. (2020) studied the impact of TiO₂-NP application on the physiological and biochemical response of wheat (*Triticum aestivum*) crop. In their findings, it was evidenced that TiO₂-NPs actively mediate the physiological and biochemical processes of wheat, which resulted in increased root/shoot length, number of tillers per plant, number of grains per plant, and grain yield along with efficient mediation of SOD, POD, MDA, and H₂O₂ activities

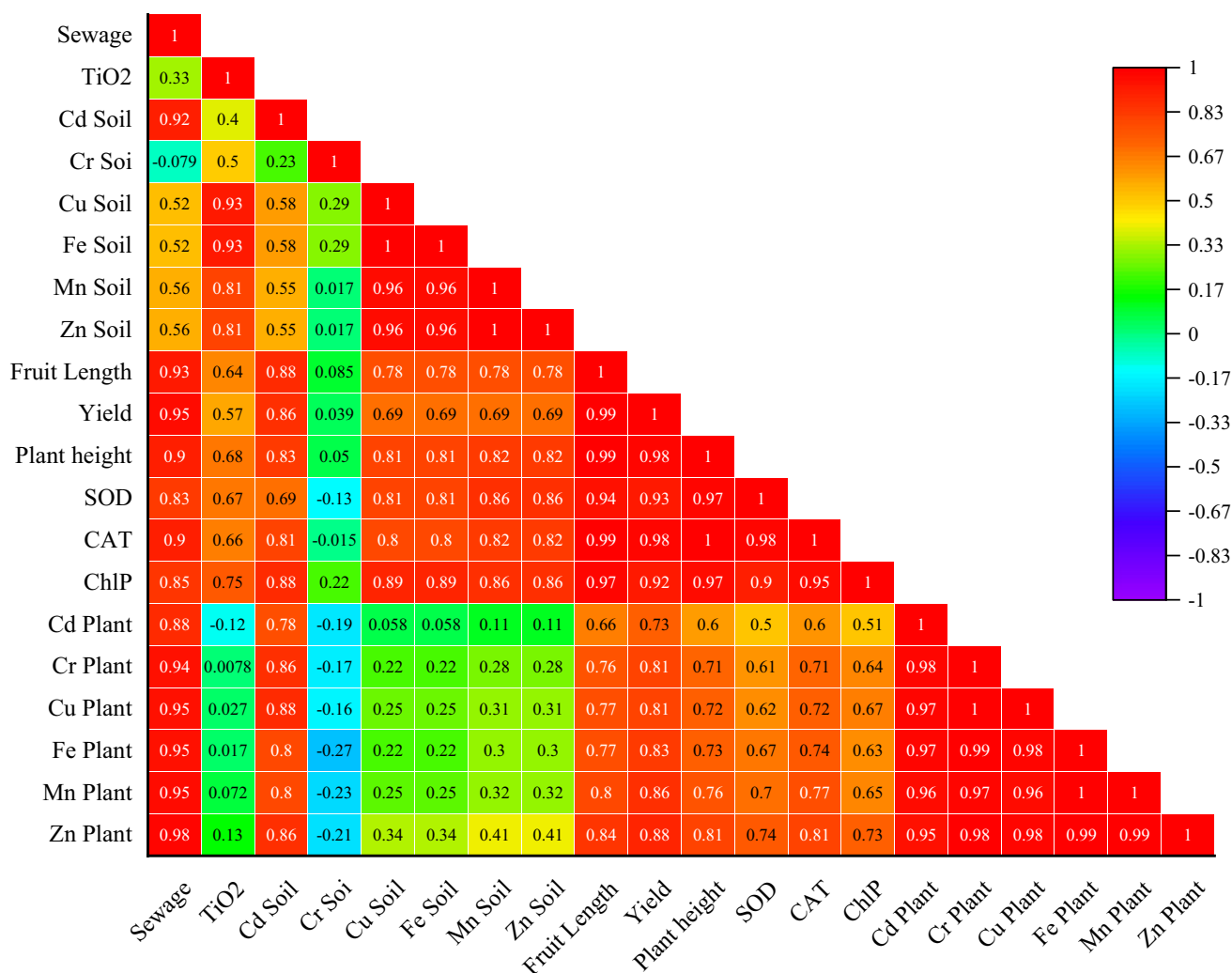


Fig. 3 Pearson correlation matrix for the effect of sewage sludge and foliar TiO₂-NP application on yield, biochemical response, and heavy metal accumulation in okra plant

best at 50 mg/kg dose. Another study by Kolenčík et al. (2020) investigated the yield response of sunflower (*Helianthus annuus*) under foliar TiO₂-NP treatments. Results indicated that the quantitative and nutritional parameters of sunflower were significantly enhanced using 2.6 mg/L TiO₂-NPs.

Effects of sewage sludge and TiO₂-NPs on heavy metal uptake

Heavy metal analysis results given in Table 3 suggested that the contents of six heavy metals (Cd, Cr, Cu, Fe, Mn, and Zn) in okra plant parts were significantly ($p < 0.05$) increased with an increase in the SS mixing rate. In general, the varying degree of heavy metal uptake was lowest in the control treatment while maximum in 100 g/kg SS treatment. However, the application of TiO₂-NPs significantly reduced the uptake of the heavy metals in both 50 and 100 g/kg treatment trials (T3 and T5). The concentration of all six heavy metals was at maximum in the root region, followed by stem and fruit parts, respectively. More specifically, the average contents of heavy metals followed the order of Fe > Mn > Cu > Zn > Cr > Cd in the whole plant tissues. The higher availability of these heavy metals leads to more

uptake by the okra plant parts; however, their uptake was slightly reduced using the foliar application of TiO₂-NPs. Correlation studies revealed that SS application showed a highly significant ($p < 0.05$) positive correlation (> 0.88) with Cd, Cr, Cu, Fe, Mn, and Zn accumulation in okra plant tissues. However, TiO₂-NP application showed neutral to negative effects in this regard (Fig. 3).

Studies on the reduction of heavy metals fate in crops using NPs have become increasingly important due to attendant soil pollution caused by rapid industrialization. Levels of Cd metal in wheat grains were significantly reduced after foliar application of TiO₂-NPs prepared using the sol–gel method (Irshad et al. 2021). Similarly, Cd accumulation was lowered in nano-TiO₂-treated cowpea (*Vigna unguiculata*) plant compared to non-treated by Ogunkunle et al. (2020). These studies reported similar outcomes that are in line with the results obtained in our study. Thus, TiO₂-NPs can be used to reduce heavy metal uptake by the okra plant.

Bioaccumulation and health risk assessment studies

Bioaccumulation of heavy metals by plants is a natural process mediated by the physiological and biochemical response of plant-root-microbe interactions (Kumar et al.

Table 3 Heavy metal accumulation in fruit, stem, roots, and full plant tissues of cultivated okra

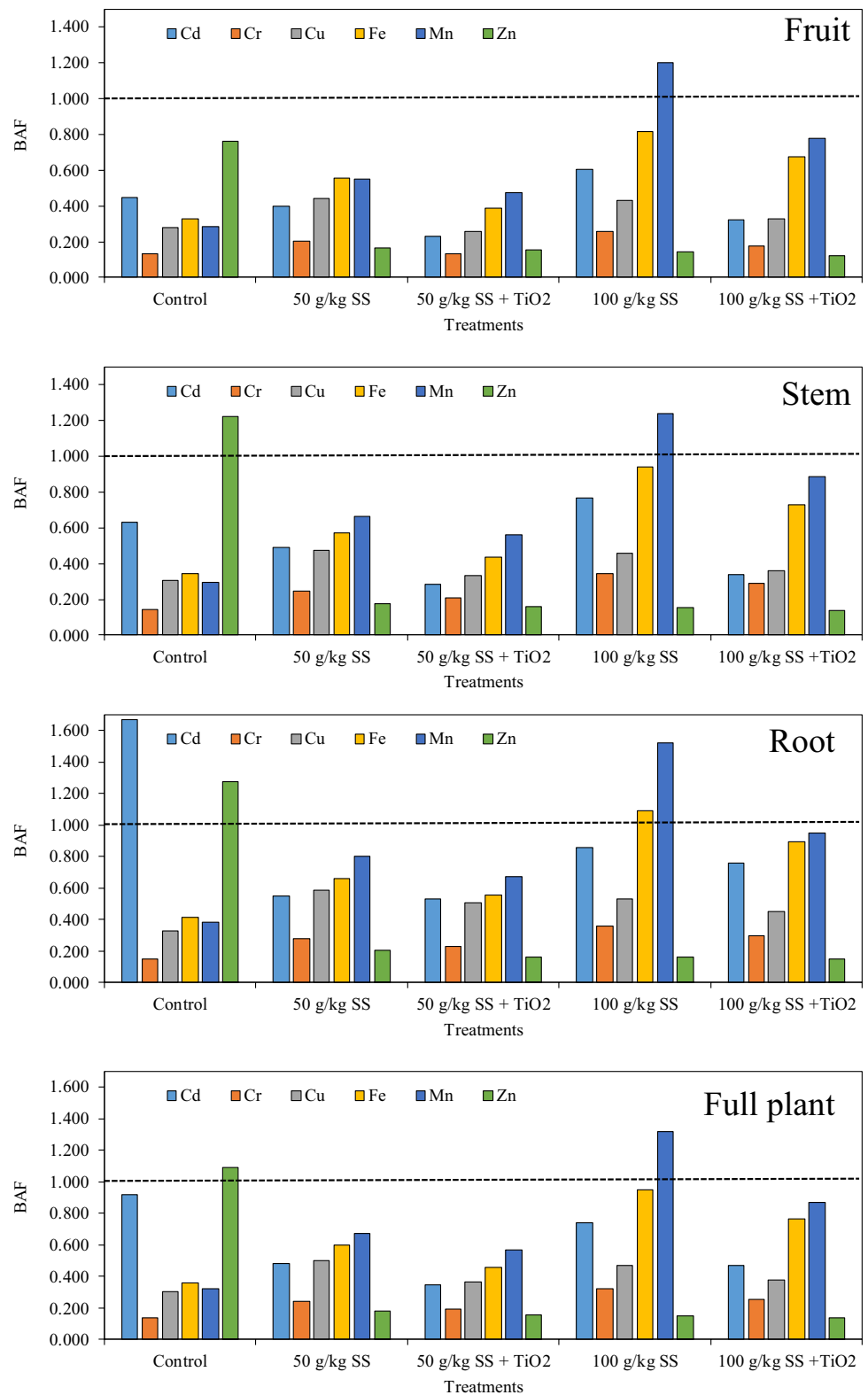
Okra tissue	Treatment	Heavy metals (mg/kg dwt.)					
		Cd	Cr	Cu	Fe	Mn	Zn
Fruit	Control	0.067 ± 0.002	0.356 ± 0.006	1.830 ± 0.005	5.981 ± 0.050	1.463 ± 0.009	2.305 ± 0.005
	50 g/kg SS	0.301 ± 0.003*	0.713 ± 0.004*	5.302 ± 0.032*	12.872 ± 0.103*	4.115 ± 0.020*	5.217 ± 0.009*
	50 g/kg SS + TiO ₂	0.176 ± 0.001*	0.464 ± 0.005*	3.109 ± 0.018*	9.004 ± 0.285*	3.560 ± 0.003*	4.881 ± 0.014*
	100 g/kg SS	0.543 ± 0.002*	1.045 ± 0.008*	8.170 ± 0.074*	21.325 ± 0.395*	10.908 ± 0.102*	8.120 ± 0.050*
	100 g/kg SS + TiO ₂	0.288 ± 0.005*	0.712 ± 0.012*	6.205 ± 0.051*	17.548 ± 0.520*	8.372 ± 0.063*	7.067 ± 0.035*
Stem	Control	0.095 ± 0.001	0.380 ± 0.015	1.993 ± 0.012	6.257 ± 0.079	1.511 ± 0.007	3.702 ± 0.020
	50 g/kg SS	0.373 ± 0.004*	0.875 ± 0.020*	5.740 ± 0.017*	13.184 ± 0.042*	5.009 ± 0.030*	5.654 ± 0.009*
	50 g/kg SS + TiO ₂	0.217 ± 0.003*	0.740 ± 0.010*	3.998 ± 0.040*	10.027 ± 0.127*	4.205 ± 0.018*	5.110 ± 0.012*
	100 g/kg SS	0.692 ± 0.011*	1.388 ± 0.016*	8.683 ± 0.031*	24.610 ± 1.060*	11.238 ± 0.078*	8.870 ± 0.018*
	100 g/kg SS + TiO ₂	0.303 ± 0.009*	1.162 ± 0.008*	6.823 ± 0.019*	19.001 ± 1.540*	8.896 ± 0.090*	8.030 ± 0.021*
Root	Control	0.250 ± 0.005	0.402 ± 0.007	2.143 ± 0.015	7.550 ± 0.031	1.948 ± 0.005	3.860 ± 0.005
	50 g/kg SS	0.419 ± 0.010*	0.986 ± 0.004*	7.055 ± 0.048*	15.320 ± 0.095*	6.018 ± 0.024*	6.572 ± 0.003*
	50 g/kg SS + TiO ₂	0.404 ± 0.014*	0.812 ± 0.003*	6.107 ± 0.029*	12.840 ± 0.070*	5.067 ± 0.082*	5.210 ± 0.007*
	100 g/kg SS	0.771 ± 0.007*	1.453 ± 0.008*	10.118 ± 0.120*	28.493 ± 1.785*	13.822 ± 0.130*	9.250 ± 0.024*
	100 g/kg SS + TiO ₂	0.685 ± 0.004*	1.205 ± 0.012*	8.559 ± 0.095*	23.370 ± 1.220*	10.510 ± 0.409*	8.626 ± 0.013*
Full plant	Control	0.137 ± 0.003	0.379 ± 0.004	1.989 ± 0.013	6.596 ± 0.016	1.641 ± 0.004	3.289 ± 0.019
	50 g/kg SS	0.364 ± 0.008*	0.858 ± 0.009*	6.032 ± 0.084*	13.792 ± 0.020*	5.047 ± 0.009*	5.814 ± 0.049*
	50 g/kg SS + TiO ₂	0.266 ± 0.004*	0.672 ± 0.010*	4.405 ± 0.113*	10.624 ± 0.055*	4.277 ± 0.006*	5.067 ± 0.107*
	100 g/kg SS	0.669 ± 0.006*	1.295 ± 0.025*	8.990 ± 0.070*	24.809 ± 0.402*	11.989 ± 0.035*	8.747 ± 0.209*
	100 g/kg SS + TiO ₂	0.425 ± 0.005*	1.026 ± 0.009*	7.196 ± 0.204*	19.973 ± 0.398*	9.259 ± 0.017*	7.908 ± 0.125*

Values are mean ± standard deviation of three replicates

SS sewage sludge

*Significantly different with garden soil as control group at $p < 0.05$

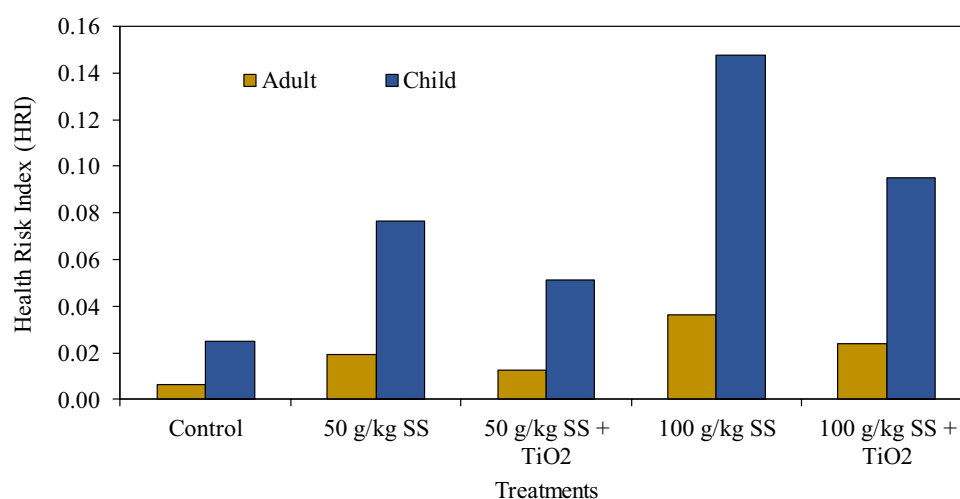
Fig. 4 Bioaccumulation factor (BAF) of heavy metals in different tissues of cultivated okra



2020a). The bioavailable heavy metals are taken by root hairs and then translocated to different plant parts such as root, shoot, stem, fruit, and leaves (Eid et al. 2021b). This biological process of accumulating heavy metals is known

as bioaccumulation. The concentration of available heavy metals in the substrate is the most determining factor that affects the rate of bioaccumulation or bioaccumulation factor (BAF). In this experiment, the root region of the

Fig. 5 Health Risk Index of heavy metals uptake by okra fruits for adult and child human groups



okra plant showed relatively higher BAF values for all six heavy metals compared to fruit and stem regions (Fig. 4). For the fruit parts of the okra plant, Cd exceeded the BAF value above 1 using a 100 g/kg SS (T4) dose, which indicated its rapid accumulation. However, it was declined below 1 using the combined 100 g/kg SS and TiO₂-NP (T5) application. Similarly, the contents of Cd, Fe, and Zn for the root region, and Cd, and Zn for stem/full plant regions exceeded the BAF > 1, which were further reduced using TiO₂-NP application in the respective treatments. The results showed that uncontrolled bioaccumulation of heavy metals can be effectively reduced using TiO₂-NP application in okra cultivation.

On the other hand, the HRI study provides information regarding the fate associated with the dietary consumption of heavy metal-contaminated vegetables and their products (Ogunkunle et al. 2020). The result indicated that the TiO₂-NPs were significantly useful in the alleviation of

SS-induced heavy metal toxicity in okra fruits. Though, the values of HRI and THQ increased with an increase in the SS dose. Overall, the child group showed greater THQ values compared to the adult group, which might be due to more susceptibility of the younger human population (Fig. 5). In this, the values of HRI and THQ did not exceed the recommended maximum limits (Kumar et al. 2021), which suggests the safe utilization of SS for okra cultivation (Table 4).

Previous studies have shown that nanoparticle application within recommended levels can enhance growth and alleviate heavy metal stress in plants (Zhou et al. 2021). Recently, Rizwan et al. (2019) found that TiO₂-NP application on the rice (*Oryza sativa*) significantly reduced the health risk of heavy metal accumulation. They found that the values of estimated BAF were lesser under TiO₂-NP application as compared to the control treatments. Moreover, Hu et al. (2020) conducted laboratory experiments for the cultivation of

Table 4 Target hazard quotient (THQ) of heavy metals in okra fruits (consumable part)

Heavy metal	Age group	THQ (mg/kg-day)				
		Control	50 g/kg SS	50 g/kg SS + TiO ₂	100 g/kg SS	100 g/kg SS + TiO ₂
Cd	Adult	0.0019	0.0089	0.0051	0.0159	0.0085
	Child	0.0085	0.0388	0.0225	0.0698	0.0370
Cr	Adult	0.0017	0.0035	0.0023	0.0051	0.0035
	Child	0.0076	0.0153	0.0100	0.0225	0.0153
Cu	Adult	0.0006	0.0019	0.0012	0.0029	0.0022
	Child	0.0005	0.0011	0.0007	0.0016	0.0011
Fe	Adult	0.0001	0.0003	0.0002	0.0004	0.0004
	Child	0.0006	0.0012	0.0008	0.0020	0.0016
Mn	Adult	0.0015	0.0043	0.0037	0.0115	0.0088
	Child	0.0067	0.0189	0.0164	0.0502	0.0385
Zn	Adult	0.0002	0.0003	0.0002	0.0004	0.0003
	Child	0.0007	0.0011	0.0010	0.0017	0.0015

coriander (*Coriandrum sativum* L.) under TiO₂-NP application. The nutrition quality of coriander was improved significantly along with a reduction in the bioaccumulation of various heavy metals. However, they suggested that a higher dose of TiO₂-NPs may damage the root system of the coriander plant. Thus, the above studies revealed that TiO₂ nanoparticle application within recommended levels could reduce the health risk of heavy metals in the okra plant.

Conclusion

The findings of this study concluded that SS mixing was helpful to increase the nutritional quality of the soil. Despite SS may bring heavy metal mixing problems in soil, still, their uptake by plants was sustainably managed through TiO₂-NP application. Overall, the combined application of SS and TiO₂-NPs increased the yield attributes and biochemical response of the okra plant while alleviating the risk of heavy metal accumulation. Various parameters including fruit length (13.97 ± 0.54 cm), yield (287.87 ± 4.06 g/plant), plant height (75.05 ± 3.18 cm), SOD (110.68 ± 3.11 µg/mg), CAT (81.32 ± 3.52 µg/mg), and chlorophyll content (3.12 ± 0.05 mg/g fwt.) reached improved levels in 100 g/kg SS and TiO₂-NP application. The health risk assessment study also indicated that the heavy metal fate was significantly reduced after the TiO₂-NP application in both child and adult groups. Further studies on the molecular understanding of root-NP interaction and speciation of accumulated heavy metals are highly recommended.

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Author contribution Pankaj Kumar: conceptualization, methodology, investigation, writing: original draft preparation, data curation, software, validation; Saad A.M. Alamri: writing: review and editing, validation; Sulaiman A. Alrumman: writing: review and editing, validation; Ebrahim M. Eid: methodology, supervision, project administration, review and editing, validation; Bashir Adelodun: software, writing: review and editing, validation; Madhumita Goala: software, writing: review and editing, validation; Kyung Sook-Choi: writing: review and editing, validation; Vinod Kumar: methodology, supervision, review and editing, resources, validation.

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Declarations

Ethics approval This article does not contain any studies with human participants or animals.

Consent to participate All the authors mutually agreed to participate in this work.

Consent for publication All the authors mutually agreed to publish the work.

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References

- Adelodun B, Ajibade FO, Ighalo JO, Odey G, Ibrahim RG, Kareem KY, Bakare HO, Tiarniyu AO, Ajibade TF, Abdulkadir TS, Adeniran KA, Choi KS (2021) Assessment of socioeconomic inequality based on virus-contaminated water usage in developing countries: a review. *Environ Res* 192:110309. <https://doi.org/10.1016/j.envres.2020.110309>
- Ali M, Niaz Y, Abbasi GH, Ahmad S, Malik Z, Kamran M, Ayaz M (2021) Exogenous zinc induced NaCl tolerance in okra (*Abelmoschus esculentus*) by ameliorating osmotic stress and oxidative metabolism. *Commun Soil Sci Plant Anal* 52(7):743–755. <https://doi.org/10.1080/00103624.2020.1869761>
- Ametepey ST, Cobbina SJ, Akpabey FJ, Duwiejuah AB, Abuntori ZN (2018) Health risk assessment and heavy metal contamination levels in vegetables from Tamale Metropolis, Ghana. *Int J Food Contam* 5:1–8. <https://doi.org/10.1186/s40550-018-0067-0>
- AOAC (2005) In: Official methods of analysis of the association of official analytical chemists. 13th edition, pp. 545–567
- AOAC (2019) Official Methods of Analysis, 21st Edition (2019) - AOAC International. <https://www.aoac.org/official-methods-of-analysis-21st-edition-2019/>. Accessed 10 Sep 2021
- APHA, AWWA, WEF (2012) Standard methods for the examination of water and wastewater, 21st Edition. American Public Health Association, Washington, pp. 2462. <https://www.standardmethods.org/>
- Chaudhary IJ, Singh V (2020) Titanium dioxide nanoparticles and its impact on growth, biomass and yield of agricultural crops under environmental stress: a review. *Res J Nanosci Nanotechnol* 10(1):1–8. <https://doi.org/10.3923/rjnm.2020.1.8>
- Chen X, Selloni A (2014) Introduction: titanium dioxide (TiO₂) nanomaterials. *Chem Rev* 114(19):9281–9282. <https://doi.org/10.1021/cr500422r>
- Cieřlik BM, Namieřnik J, Konieczka P (2015) Review of sewage sludge management: standards, regulations and analytical methods. *J Clean Prod* 90:1–15. <https://doi.org/10.1016/j.jclepro.2014.11.031>
- da Silva WR, do Nascimento CWA, da Silva FBV, de Souza AAB, Fracetto GGM, Ximenes DHDSV (2021) Effects of sewage sludge stabilization processes on soil fertility, mineral composition, and grain yield of maize in successive cropping. *J Soil Sci Plant Nutr* 21(2):1076–1088. <https://doi.org/10.1007/s42729-021-00423-1>
- Eid E, Alrumman SA, El-Bebany AF, Fawy KF, Taher MA, Hesham AEL, Ahmed MT (2019) Evaluation of the potential of sewage sludge as a valuable fertilizer for wheat (*Triticum aestivum* L.) crops. *Environ Sci Pollut Res* 26(1):392–401. <https://doi.org/10.1007/s11356-018-3617-3>
- Eid E, Alrumman SA, El-Bebany AF, Fawy KF, Taher MA, Hesham AEL, Ahmed MT (2018) The evaluation of sewage sludge application as a fertilizer for broad bean (*Faba sativa* Bernh.) crops. *Food Energy Secur* 7(3):e00142. <https://doi.org/10.1002/fes3.142>
- Eid EM, Kumar P, Adelodun B, Choi KS, Singh J, Kumari S, Kumar V (2021a) Modeling of mineral elements uptake and localization in cabbage inflorescence (*Brassica oleracea* var. capitata) grown on sugar mill pressmud-amended soils. *Environ Monit Assess* 193(9):1–13. <https://doi.org/10.1007/s10661-021-09381-8>

- Eid EM, Shaltout KH, Alamri SAM (2021b) Monitored sewage sludge application improves soil quality, enhances plant growth, and provides evidence for metal remediation by *Sorghum bicolor* L. J Soil Sci Plant Nutr 21:2325–2338. <https://doi.org/10.1007/s42729-021-00524-x>
- EUROSTAT (2014) Environment data base. <http://epp.eurostat.ec.europa.eu/portal/page/portal/environment/data/database>. Accessed 07 December 2021
- Goala M, Yadav KK, Alam J, Adelodun B, Choi KS, Cabral-Pinto MMS, Hamid AA, Alhoshan M, Ali FAA, Shukla AK (2021) Phytoremediation of dairy wastewater using *Azolla pinnata*: application of image processing technique for leaflet growth simulation. J Water Process Eng 42:102152. <https://doi.org/10.1016/j.jwpe.2021.102152>
- Hong J, Wang C, Wagner DC, Gardea-Torresdey JL, He F, Rico CM (2021) Foliar application of nanoparticles: mechanisms of absorption, transfer, and multiple impacts. Environ Sci Nano 8(5):1196–1210. <https://doi.org/10.1039/D0EN01129K>
- Hu J, Wu X, Wu F, Chen W, White JC, Yang Y, Wang X (2020) Potential application of titanium dioxide nanoparticles to improve the nutritional quality of coriander (*Coriandrum sativum* L.). J Hazard Mater 389:121837. <https://doi.org/10.1016/j.jhazmat.2019.121837>
- Hube S, Wu B (2021) Mitigation of emerging pollutants and pathogens in decentralized wastewater treatment processes: a review. Sci Total Environ 779:146545. <https://doi.org/10.1016/j.scitotenv.2021.146545>
- Irshad MA, Ur Rehman MZ, Anwar-ul-Haq M, Rizwan M, Nawaz R, Shakoor MB, Ali S (2021) Effect of green and chemically synthesized titanium dioxide nanoparticles on cadmium accumulation in wheat grains and potential dietary health risk: a field investigation. J Hazard Mater 415:125585. <https://doi.org/10.1016/j.jhazmat.2021.125585>
- Jampilek J, Kráľová K (2015) Application of nanotechnology in agriculture and food industry, its prospects and risks. Ecol Chem Eng S 22(3):321–361. <https://doi.org/10.1515/eces-2015-0018>
- Kolenčík M, Ernst D, Urík M, Ďurišová L, Bujdoš M, Šebesta M, Kratošová G (2020) Foliar application of low concentrations of titanium dioxide and zinc oxide nanoparticles to the common sunflower under field conditions. Nanomater 10(8):1619. <https://doi.org/10.3390/nano10081619>
- Kumar V, Kumar P, Khan A (2020a) Optimization of PGPR and silicon fertilization using response surface methodology for enhanced growth, yield and biochemical parameters of French bean (*Phaseolus vulgaris* L.) under saline stress. Biocatal Agric Biotechnol 23:101463. <https://doi.org/10.1016/j.bcab.2019.101463>
- Kumar V, Kumar P, Singh J, Kumar P (2020b) Potential of water fern (*Azolla pinnata* R. Br.) in phytoremediation of integrated industrial effluent of SIIDCUL, Haridwar, India: removal of physicochemical and heavy metal pollutants. Int J Phytoremediation 22(4):392–403. <https://doi.org/10.1080/15226514.2019.1667950>
- Kumar V, Kumar P, Singh J, Kumar P (2021) Use of sugar mill wastewater for *Agaricus bisporus* cultivation: prediction models for trace metal uptake and health risk assessment. Environ Sci Pollut Res 28(21):26923–26934. <https://doi.org/10.1007/s11356-021-12488-7>
- Kumar V, Eid EM, Al-Bakre DA, Abdallah SM, Širić I, Andabaka Ž, Kumar P, Goala M, Adelodun B, Singh J, Kumari S, Bachheti A, Arya AK, Choi K-S (2022) Combined Use of Sewage Sludge and Plant Growth-Promoting Rhizobia Improves Germination, Biochemical Response and Yield of Ridge Gourd (*Luffa acutangula* (L.) Roxb.) under Field Conditions. Agric 12(2):173. <https://doi.org/10.3390/agriculture12020173>
- Li S, Zhang K, Zhou S, Zhang L, Chen Q (2009) Use of dewatered municipal sludge on Canna growth in pot experiments with a barren clay soil. Waste Manag 29(6):1870–1876. <https://doi.org/10.1016/j.wasman.2008.12.007>
- Lin X, Li S, Wei Z, Chen Y, Hei L, Wu QT (2021) Indirect application of sludge for recycling in agriculture to minimize heavy metal contamination of soil. Resour Conserv Recy 166:105358. <https://doi.org/10.1016/j.resconrec.2020.105358>
- Malik OA, Hsu A, Johnson LA, de Sherbinin A (2015) A global indicator of wastewater treatment to inform the sustainable development goals (SDGs). Environ Sci Policy 48:172–185. <https://doi.org/10.1016/j.envsci.2015.01.005>
- Mara D (2013) Domestic wastewater treatment in developing countries. Earthscan Publications, Routledge, p 311
- Mittal D, Kaur G, Singh P, Yadav K, Ali SA (2020) Nanoparticle-based sustainable agriculture and food science: recent advances and future outlook. Front Nanotechnol 2:10. <https://doi.org/10.3389/fnano.2020.579954>
- Ogunkunle CO, Odulaja DA, Akande FO, Varun M, Vishwakarma V, Fatoba PO (2020) Cadmium toxicity in cowpea plant: effect of foliar intervention of nano-TiO₂ on tissue Cd bioaccumulation, stress enzymes and potential dietary health risk. J Biotechnol 310:54–61. <https://doi.org/10.1016/j.jbiotec.2020.01.009>
- Pachura P, Ociepa-Kubicka A, Skowron-Grabowska B (2016) Assessment of the availability of heavy metals to plants based on the translocation index and the bioaccumulation factor. Desalin Water Treat 57(3):1469–1477. <https://doi.org/10.1080/19443994.2015.1017330>
- Panpatte DG, Jhala YK, Shelat HN, Vyas RV (2016) Nanoparticles: the next generation technology for sustainable agriculture. Microbial inoculants in sustainable agricultural productivity. Springer, New Delhi, pp 289–300
- Pestovsky YS, Martínez-Antonio A (2017) The use of nanoparticles and nanoformulations in agriculture. J Nanosci Nanotechnol 17(12):8699–8730. <https://doi.org/10.1166/jnn.2017.15041>
- Qian L, Wang S, Xu D, Guo Y, Tang X, Wang L (2016) Treatment of municipal sewage sludge in supercritical water: a review. Water Res 89:118–131. <https://doi.org/10.1016/j.watres.2015.11.047>
- Rico CM, Majumdar S, Duarte-Gardea M, Peralta-Videa JR, Gardea-Torresdey JL (2011) Interaction of nanoparticles with edible plants and their possible implications in the food chain. J Agric Food Chem 59(8):3485–3498. <https://doi.org/10.1021/jf104517j>
- Rizwan M, Ali S, Ur Rehman MZ, Malik S, Adrees M, Qayyum MF, Ahmad P (2019) Effect of foliar applications of silicon and titanium dioxide nanoparticles on growth, oxidative stress, and cadmium accumulation by rice (*Oryza sativa*). Acta Physiol Plant 41(3):1–12. <https://doi.org/10.1007/s11738-019-2828-7>
- Rodrigues Prates A, Renée Coscione A, Carvalho Minhoto Teixeira Filho M, Gasparoti Miranda B, Arf O, Hamilton Abreu-Junior C, Assis Rodrigues Nogueira T (2020) Composted sewage sludge enhances soybean production and agronomic performance in naturally infertile soils (Cerrado Region, Brazil). Agronomy 10(11):1677. <https://doi.org/10.3390/agronomy10111677>
- Sabeen AH, Noor ZZ, Ngadi N, Almuraisi S, Raheem AB (2018) Quantification of environmental impacts of domestic wastewater treatment using life cycle assessment: a review. J Clean Prod 190:221–233. <https://doi.org/10.1016/j.jclepro.2018.04.053>
- Sato T, Qadir M, Yamamoto S, Endo T, Zahoor A (2013) Global, regional, and country level need for data on wastewater generation, treatment, and use. Agric Water Manag 130:1–13. <https://doi.org/10.1016/j.agwat.2013.08.007>
- Shahid M, Farooq ABU, Rabbani F, Khalid S, Dumat C (2020) Risk assessment and biophysiochemical responses of spinach to foliar application of lead oxide nanoparticles: a multivariate analysis. Chemosphere 245:125605. <https://doi.org/10.1016/j.chemosphere.2019.125605>
- Shaltout KH, Alamri SA, Alrumman SA, Hussain AA, Sewelam N, Eid EM (2021) Evaluation of uptake of eight metals by *Sorghum bicolor* grown in arable soil combined with sewage sludge based on prediction models. Environ Monit Assess 193(8):1–15. <https://doi.org/10.1007/s10661-021-09320-7>

- Tagg AS, Brandes E, Fischer F, Fischer D, Brandt J, Labren M (2021) Agricultural application of microplastic-rich sewage sludge leads to further uncontrolled contamination. *Sci Total Environ* 150611:1–10. <https://doi.org/10.1016/j.scitotenv.2021.150611>
- Tyła M (2019) Assessment of heavy metal pollution and potential ecological risk in sewage sludge from municipal wastewater treatment plant located in the most industrialized region in Poland-case study. *Int J Environ Res Public Health* 16(13):2430. <https://doi.org/10.3390/ijerph16132430>
- Ullah S, Adeel M, Zain M, Rizwan M, Irshad MK, Jilani G, Rui Y (2020) Physiological and biochemical response of wheat (*Triticum aestivum*) to TiO₂ nanoparticles in phosphorous amended soil: A full life cycle study. *J Environ Manag* 263:110365. <https://doi.org/10.1016/j.jenvman.2020.110365>
- UNWWR (2021) United Nations World Water Report. Progress on Wastewater Treatment - 2021 Update. IMI-SDG6 SDG 6 PROGRESS REPORTS. Available online at: <https://www.unwater.org/publications/progress-on-wastewater-treatment-631-2021-update/>
- USEPA (2011) Integrated Risk Information System, USEPA. <https://www.epa.gov/iris>. Accessed 18 November 2021
- Walkley A, Black IA (1934) An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci* 37:29–38
- Yakamercan E, Ari A, Aygün A (2021) Land application of municipal sewage sludge: human health risk assessment of heavy metals. *J Clean Prod* 319:128568. <https://doi.org/10.1016/j.jclepro.2021.128568>
- Zhou P, Adeel M, Shakoor N, Guo M, Hao Y, Azeem I, Rui Y (2021) Application of nanoparticles alleviates heavy metals stress and promotes plant growth: an overview. *Nanomater* 11(1):26. <https://doi.org/10.3390/nano11010026>
- Zuo W, Bai Y, Lv M, Tang Z, Ding C, Gu C, Li M (2021) Sustained effects of one-time sewage sludge addition on rice yield and heavy metals accumulation in salt-affected mudflat soil. *Environ Sci Pollut Res* 28(6):7476–7490. <https://doi.org/10.1007/s11356-020-11115-1>

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