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Research Article

A study on the evaluation of heavy metals accumulation in electrokinetically treated sewage sludge by *Spinacia oleracea*

Tamanna Parida
Department of Environmental Science, GITAM School of Science, GITAM, (Deemed to be University), Visakhapatnam (Andhra Pradesh), India
Ram Sailesh Agnihotri
Department of Environmental Science, GITAM School of Science, GITAM, (Deemed to be University), Visakhapatnam (Andhra Pradesh), India
Suresh Kumar Kolli
Department of Environmental Science, GITAM School of Science, GITAM, (Deemed to be University), Visakhapatnam (Andhra Pradesh), India
Anindita Chakraborty
UGC DAE, Consortium for Scientific Research, Kolkata Centre (West Benga)l, India
Namuduri Srinivas*
Department of Environmental Science, GITAM School of Science, GITAM, (Deemed to be University), Visakhapatnam (Andhra Pradesh), India

*Corresponding author. E-mail: snamudur@gitam.edu

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Abstract
The sewage sludge (SS) profile combines potentially toxic metals and organic matter that help nourish the soil in many ways. Still, toxicity raises concerns about the contamination of the food chain and pollution of the soil, water, and air. Treatment of raw SS by physical and chemical methods is a challenging task with a big budget and does not support the sustainable approach. On the other hand, phytoremediation coupled with electrokinetic treatment treats the raw sludge by extracting the maximum amount of heavy metals (HMS) and enhancing its quality by improving the physicochemical parameters. The core study of this paper is to determine the accumulation of heavy metals from EKT SS by *Spinacia oleracea*. Two setups were prepared by amending the SS with garden soil; one (untreated) was directly subjected to phytoremediation, whereas EKT influenced the other for 11 days and allowed the plant to grow (treated). Results have shown that the extraction of Pb and Zn was high in both sets without compromising the plant's metabolism. EKT encourages the organic carbon, pH, and conductivity of the SS and promotes the growth of the plant in comparison to the untreated setup. EKT made all elements highly available, helping plants to absorb some high elements efficiently, and some elements, such as As and Cr, reported the lowest extraction. Pb is known for its high toxicity, but Spinach could absorb more than the range by increasing its stress tolerance index.

Keywords: Bioaccumulation factors, Electro-Kinetic treatment, Heavy metals, Hyperaccumulator, Sewage sludge

INTRODUCTION

Automobiles, industries, domestic, medical, and agricultural practices are the major sectors that contribute heavy metals (HMs) to the environment through the input and output of inorganic material (Srivastava *et al.,*2017). Implementation of HMs such as Cadmium (Cd), Cobalt (Co), Copper (Cu), Chromium (Cr), Mercury (Hg), Arsenic (As), Nickel (Ni), and Iron (Fe) in different industries is an intensive practice for the high production to meet the needs of the population. However, it comes at a price to compromise the biota of the ecosystem. It directly or indirectly contaminates the fertile land and interferes with the food chain, compromising animal and human health (Scutarașu and Trincă, 2023; Yedjou *et al.,*2012). Many industries depend on advanced renewable technologies that seek HMs such as Pd, Ni, and Cd, which are used for batteries of electric cars, solar cells, and battery cells to claim sustainable energy (Hou *et al.,*2022).
On the other hand, a constantly rising population has a high demand for food, leading to aggressive agriculture by using high amounts of pesticides and fertilizer, another primary reason for HM contamination in air, soil, and water. These derived chemicals left a large number of undegradable HMs in the soil that were ultimately

absorbed by the next batch of crops as HMs do not degrade but rather transform from one organic or oxidation state to another (Rashid *et al.,*2023; Ciont *et al.,*2022; Garbisu and Alkorta, 2001). These are the principal sectors that introduce HMs into the environment, and their waste indulges with high amounts of toxins that are reflected in the composition of Sewage Sludge (SS).

SS is the by-product that comes after the secondary waste from the waste treatment plant (Srinivas *et al.,*2022). It has a complex feature as it is rich in organic content and dominant with HMs. SS is composed of 1.2-3% total phosphorous, 2.8–4.9% total nitrogen, 20.5 - 40.3% organic carbon, and total potassium < 1% (Collivignarelli *et al.* 2019); at the same time, an excess amount of HMs makes difficulties of its direct use in any field. SS disposal should be taken care of as it can contaminate the soil, water, and air (Rosiek, 2020; Saha *et al.,*2017).

Proper treatment of this massive waste is required to decrease toxicity and avoid contaminating the food chain to save human and animal health. Three classes, physical, chemical, and biological treatments, are there to treat the waste with different efforts (Manoj *et al.,*2020; Ulla *et al.,*2019). Out of these, physical and chemical procedures are expensive, need more attention, and emit secondary pollutants that may alter the soil properties. In chemical treatment, chemicals are leached off to the soil and disturb the soil configuration as minerals are lost, microbial colonies are interrupted, and human power is sought at a high cost. On the other hand, physical methods, like the membrane filtering process, which have drawbacks such as membrane fouling issues, limited lifespans, and the rate of periodic replacement (Dos Santos *et al*., 2007; Forgacs *et al*., 2004). In cost-effective analysis, physical and chemical remediation is on the negative side as they demand more expenses more often and energy use is high. There is a high chance of further soil water and air contamination.

Amid these problems, phytoremediation from biological treatment comes up with a sustainable approach that offers multiple benefits. Plants are used in phytoremediation to clean up contaminated media, such as soil and water. Plants that have high-stress tolerance capacity are generally used for this remediation process, an environmentally and economically beneficial method of containing, sequestering, or detoxifying pollutants from contaminated soil and water. To degrade, remove, or immobilize the contaminants, phytoremediation employs a variety of mechanisms, such as degradation (rhizome-degradation, phytodegradation), accumulation (phytoextraction and rhizofiltration), dissipation (phytovolatilization), and immobilization (hydraulic control and phytostabilization). Plants use one or more of these methods to lower the amounts of pollutants in soil and water, depending on the type of contaminant. For instance, plants absorb heavy metals (HMs) and store them in their tissues. They also break down organic contaminants in soil and water, lessening their toxicity (Shah and Daverey, 2020; Pivetz, 2001a). Several plant species were identified as hyperaccumulators belonging to families of Violaceae, Euphorbiaceae, Conouniaceae, Fabacceae, Caryophyllaceae, Lamiaceae, Brassicaceae, etc. Out of all, more than 500 plants from Brassicaceae dominate the phytoremediation process (Sarma, 2011). It allows native plants to grow and contributes to carbon sequestration. Some plants possess high biomass, which helps in terms of bioenergy after harvesting. However, the downside of this process is that plants can only absorb bioavailable metals in the soil as well as takes time to remediate (Zhong *et al.,*2015; Gavrilescu, 2022).

In contrast, most of the HMs are less available in the soil, leading to a less effective or incomplete remediation process. On the other hand, electrokinetic treatment (EKT) helps to make HMs bioavailable with the help of electricity. Low voltage direct current is employed with electrodes are put into contaminated sewage sludge and the current is passed through them. According to Fu *et al*. (2017), this generates an electric field and leads to metal migration towards the electrodes that serve as the anode and cathode. The kind of metal species present, their concentration, ion mobility, soil composition, moisture content, solution chemistry, and other elements all significantly impact HMs migration. It generally manipulates the electrons, which helps to mobile them to their respective electrodes (anode or cathode) through the electro-osmosis or electrophoresis process. It accumulates metals that help absorb more toxins in a limited period (Cameselle and Reddy, 2012). According to Wang *et al*. (2018), the EKT process involves three primary mechanisms: electromigration, electroosmosis, and electrophoresis. Ions move towards the oppositely charged electrodes in electromigration (Fu *et al*. 2017; Zhu *et al*. 2015), while in electroosmosis, the electric field's difference results in fluid transport via the pores in the sludge or soil. Electrophoresis is used to transport charged particles or ions due to an external electric current. *Spinacia oleracea* from the family of Caryophyllales is considered a test species in this study as this leafy vegetable has a wide leaf surface with a fast growth rate in a short period. The population widely consumes this vegetable due to its nutritional value. This study has considered Spinach as a test species for the absorption of HMs from electrokinetically treated sludge amended with garden soil that helps to evaluate spinach's bioaccumulation potential. Less maintenance of the species, quick adaptation to environmental changes, and shorter life spans help minimize the time consumption taken by the phytoremediation process. The present study aimed to evaluate the bioaccumulation potential of *Spinacia oleracea* using electrokinetically treated sewage sludge.

MATERIALS AND METHODS

Sampling and analysis of sludge

The electro-phyto remediation experiment was conducted at GITAM (Deemed to be University), Visakhapatnam, Andhra Pradesh, with a latitude of 17.7816° N and a longitude of 83.3775° E. The raw sewage sludge (SS) sample was collected from the Appu Ghar Sewage Treatment plant, Vishakhapatnam. It is located on the coast of the Bay of Bengal and treats municipal solid waste collected from Vishakhapatnam. pH, organic carbon (OC), and conductivity were analyzed using unprocessed SS. It was homogenized and sun-dried for 3-4 days. The experiment was accomplished in two steps: EKT and phytoremediation and the whole procedure has been portrayed in Fig.1.

Electro-kinetic treatment setup

A 4:1 ratio between water and SS was maintained in 20 liters of two glass chambers with dimensions of 37 x 33.5 x 25 cm (LxDxH) with 6 mm in thickness. Medium-sized granule SS were completely dried and occupied more space and a slurry form was required to flow the fluent electricity. So, the 4:1 ratio was maintained in the glass chambers. One was mentioned as treated (with EK treatment), and another one was left without any treatment (untreated). Two stainless steel sheets, 8" x 8", were subjected to both ends of the treated chamber as anode and cathode, with the help of crocodile clips that were connected to the regulated power supply. 10V (DC) was supplied to the chamber for 6 hours every day, and this process was repeated for 11 days, whereas the DC did not influence the untreated one. SS was allowed to be sun-dried for further procedures after the accomplishment of EK treatment (Srinivas *et al.,*2023).

Greenhouse setup

Two batches of treated and untreated SS were prepared by mixing red garden soil, maintaining 10, 25, and 50% concentrations to increase SS quantity in soil from low to high. 5 kg pots were taken for the phytoremediation, and four sets (two sets for untreated and two sets for treated) were maintained throughout the experiment, whereas one separate pot was filled with only red garden soil for comparison purposes. 500 grams of SS with 4.5 kgs of soil was taken for 10% concentration, followed by1.25 kgs of SS with 3.75 kgs of soil for 25% and 2.5 kgs of SS with 2.5 kgs of soil for 50% for untreated setup and the SS used for this was not influenced by the EKT. The same method was also set up for treatment with the EKT-influenced SS. Twenty-five seeds of *S. oleracea* were sowed in each pot for 51 days. Soil and plant samples were allowed for sun-dry in a post-harvesting period. An electronic weighing balance meter measured dry biomass, and then dried plants and soil were grounded and passed through a 1 mm sieve for further analysis.

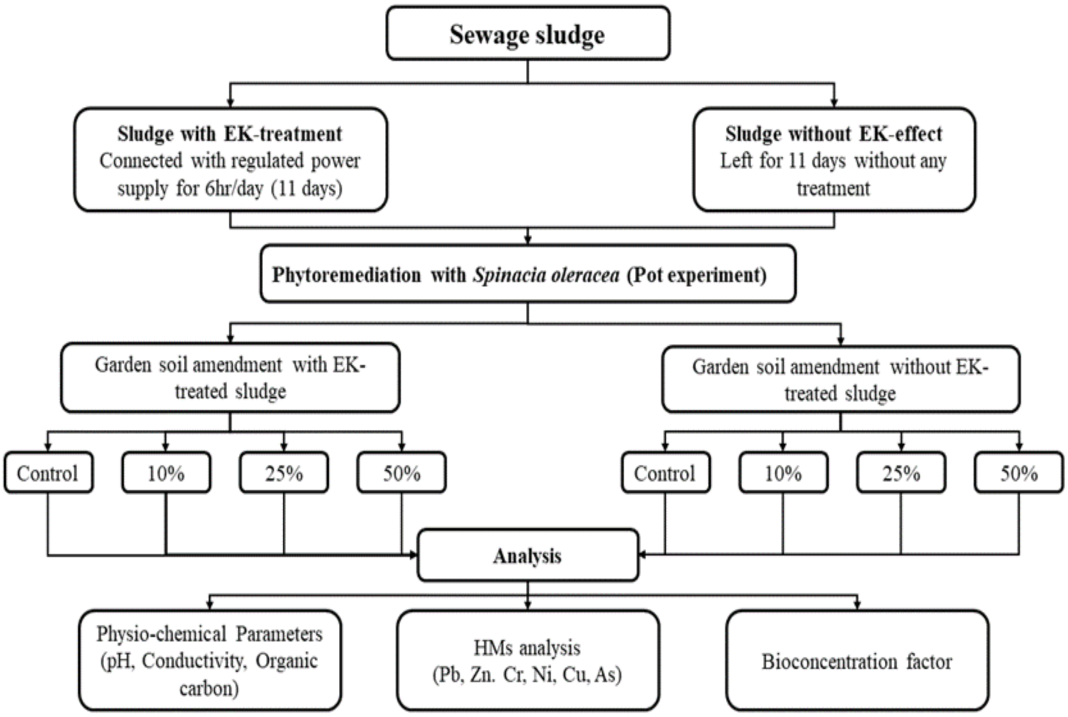

Fig. 1. *Schematic representation of Phyto-Electrokinetic treatment to Spinacia oleracea*

Estimation of heavy metals in plant and soil by ED-XRF

Energy dispersive X-ray fluorescence (ED-XRF) was used to estimate HMs in both plant and soil samples. Duplicate pellets were prepared to minimize the error percentage. Pellets were placed in different plastic containers and kept inside the ED-XRF for the analysis (Srinivas *et al.,*2023).

Assessment of physicochemical parameters in soil and sludge samples

pH, organic carbon (OC), and conductivity have been handled under physicochemical parameters. SS was dissolved in distilled water with a ratio of 1:5 after 5mins pH and conductivity readings were recorded using their respective meters measurements. This standard procedure for soil was followed as cited in FAO (2021). OC was done using the titration method following the procedure of Walkley and Black (1934).

Bioaccumulation factor

The bioaccumulation factor (BAF) is applied to determine plants' total absorption of HMs from the contaminated soil. Using the formula, this index helps calculate plant absorption of specific metals to the soil concentration (Chen *et al.,*2023).

BAF = Metal concentration in the whole plant / Metal concentration in the soil Eq.1

Biomass and Stress Tolerance Index

Plants of both sets and control plants, were uprooted on their final day and allowed for sundry. After a week, the dry weight of the whole plant was weighed by an electronic weighing balance meter and compared with the control plants. Biomass plays an important role in assessing the stress tolerance index of a plant by using the following formula (Gardea-Torresdey *et al.,*2004).

STI = Dry weight of (treated or untreated plant) / Dry weight of control plant ×100 Eq-2

Determining surface morphology and structural composition by FE- SEM/EDAX

The structure and composition of SS were done using the field emission scanning electron microscopy with energy dispersive X-ray spectroscopy (FE-SEM/EDX) model of TESCAN MIRA. The powder samples were mounted on the carbon tape of the standard pin and then coated with 10-20 nm thickness of gold by sputter coater in 90 seconds with 12 pa pressure. FE-SEM captured the surface structure, while EDX determined the elemental composition (Smith *et al.,*2009; TESCAN, 2023).

Statistical analysis

Analysis of variance (ANOVA)

ANOVA is performed to recognize the variation between considered parameters in a specified database. A significant variable (dependent variable) in the database is allowed to observe the sources of variation in the values. With this method, a significant difference in the variable that occurs or does not occur can be identified. In this study, single-factor ANOVA has been performed (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

Physiochemical parameters

The three physicochemical parameters, pH, EC, and OC, were considered, and values are tabulated in Table 1. The pH of untreated soil was mildly acidic and varied from 6.6 to 6.8, whereas in the case of the treated setup, pH enhanced from acidic to slightly alkaline and varied from 7.3 to 7.6. The EC of the treated setup was progressive from lower concentration to higher concentration (0.6 to 1 mS cm^{-1}), which indicates the availability of ions boosted after the EK treatment. In contrast, the untreated setup showed lesser EC (0.3 to 0.6 mS cm^{-1}). Srinivas *et al*(2023), Wong *et al* (1997), and Wang *et al.* (2021) found the same trend as pH and EC encouraged after applying EK treatment to sludge and soil amended with sodium alginate, respectively. OC of the treated setup was quite impressive as readings rose from 3.1 to 4.4%, while the untreated setup varied from 0.9 to 1.3%. Enhancement of OC was observed in both setups, but due to the EK effect, the treated group showed higher results. OC discourages bulk density, which implies high aggregated stability. Inorganic nutrients and OC help to nourish the soil. Steady improvement of OC facilitates an environ-

Table 1. Values (Mean and ± Standard deviation) of physicochemical parameters and Stress Tolerance Index (STI) of *Spinach olearecea*

Amends (%)	pH		Conductivity (µS/m)		Organic carbon (%)		STI	
	Untreated SS	Treated SS	Untreated SS	Treated SS	Untreated SS	Treated SS	Untreated SS	Treated SS
Control	6.5±0.21	--	0.3±0.10	--	1.8±0.31	--	--	--
10	6.6±0.15	7.3±0.06	0.3±0.15	0.6±0.07	0.9±0.64	3.1±7	119.78	154.94
25	6.5±0.06	7.4±0.10	0.5±0.12	0.7±0.14	1.1±0.64	4.3±29	134.79	160.43
50	6.8±0.12	7.6±0.12	0.6±0.10	1±0.01	1.3±0.92	4.4±47	102.19	127.83

ment for microbes by mineralizing the organic matter (biodegradability of OC) in the system (Ojeda *et al*., 2003; Morera *et al.,*2002) and the same trends were found by Garcıa-Gil *et al.,* (2000); Frąc *et al.* (2012) in organic manure and fertilizer soils. SS has a low pH due to the presence of fatty acids that reduce the growth of methanogenic bacteria, which causes problems in agriculture by altering soil properties. From this study, pH enhanced from acidic to neutral after EK treatment, supporting microbial growth. It improves the organic matter and ion concentration reflected in OC and EC readings (Singh *et al.,*2020).

Morphological study of sewage sludge and phyto-accumulation of HMs by *Spinacia oleracea*

Two stages of samples, intermediate (6th) and final (11th) day of both untreated and treated setup, were collected for characterization of the SS surface through micro-scanning of FE-SEM/EDAX. It is porous in nature with many granular structures that indicate the water retention potential of SS (Wang *et al.,*2014). On both days (6th and 11th), treated samples revealed more amorphous structures than the untreated setup; big chunky particles broke down into small granules that can easily spread on the ground and act as a plant water capsule (Fig. 2). The ED-XRF analysis has further confirmed compositional changes. The heterogenous and porous structure of SS was also found by Acharya *et al*(2021).

The core focus of the experiment is to estimate the sequestration of HMs from sewage-amended soil by the test species (*Spinacia oleracea*) after EK treatment. Two significant segments, soil and plant (root + shoot), have been considered for ED-XRF analysis. Six HMs, such as Cr, As, Pb, Ni, Cu, and Zn were estimated in both segments, and values are recorded in Table 2.

In the case of Cr, the treated setup absorbed quite less than the untreated setup. 10% concentration of untreated setup absorbed 38.53 ppm followed by 39.09 and 58.53 for 25 and 50%, while treated setup absorbed 33.57 ppm followed by 30.9 and 28.59 ppm for 25 and 50%, respectively. However, soil maintained a different trend as untreated soil has less values than the treated soil except for 50%, indicating enhanced Cr availability after EK treatment that affected the Cr absorption. A higher concentration of Cr in soil limits the nutrient up-

A

B

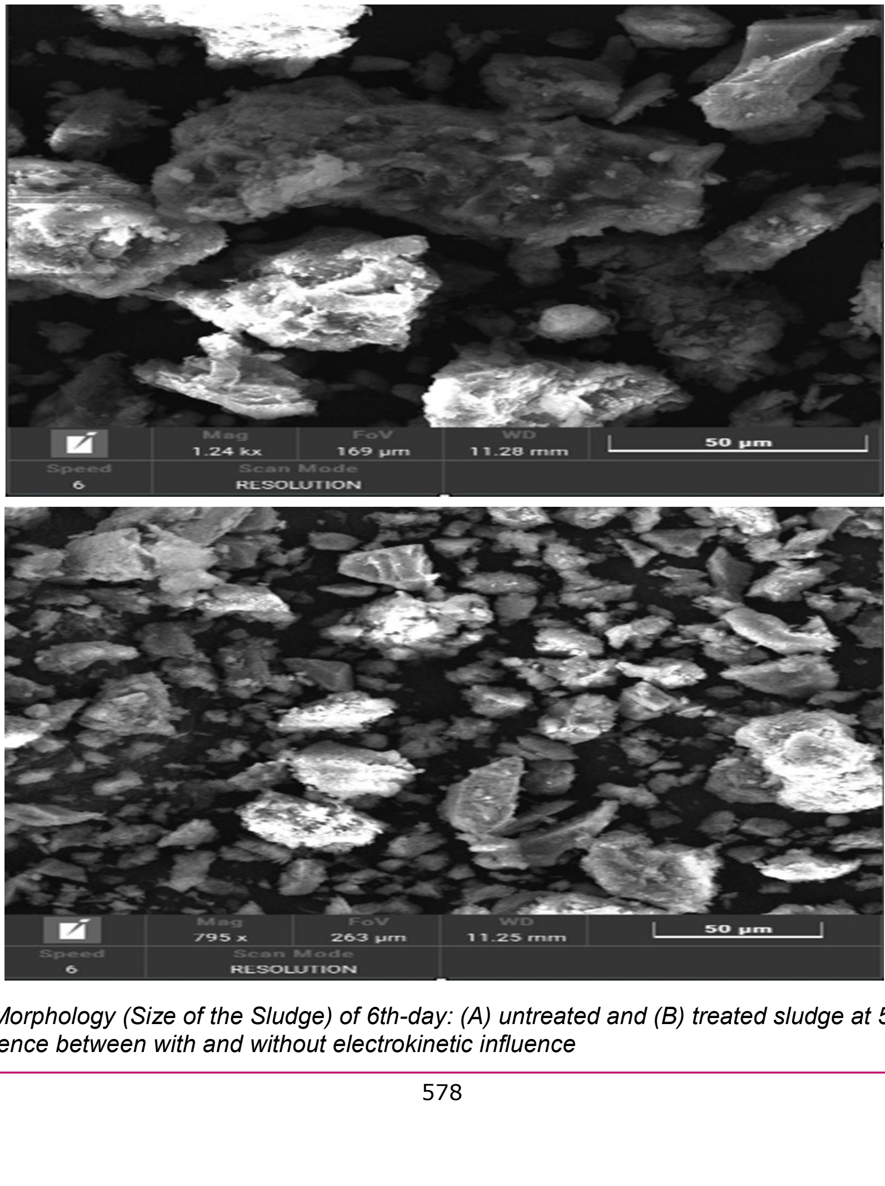

Fig. 2. (A & B). *Morphology (Size of the Sludge) of 6th-day: (A) untreated and (B) treated sludge at 50µm with 25 KeV portrays the difference between with and without electrokinetic influence*

take of plants by forming insoluble compounds (Osu Charles and Onyema, 2016). As followed the same path of Cr as As absorption by the treated setup was again low compared to the untreated setup.in 10%, treated reported 0.35 ppm while untreated reported 0.38 ppm whereas 25% showed a major difference as treated reported 0.29 ppm and untreated reported 0.49 ppm respectively however 50% (0.39 ppm) of untreated reduced. On the other hand, the values of treated soil were somewhat less than those of untreated soil, but in 50%, values increased from 7.58 ppm (untreated) to 8.12 ppm (treated). Arsenic (As) beyond the permissible limit can harm plants, including stunted roots, withering leaves, decreased amounts of photosynthetic pigment, yellowing of the leaves, and a reduction of chlorophyll (Chl), which can alter plant metabolism (Abbas *et al*., 2018).

Cu, Pb, Ni, and Zn are in one trend line as absorption in the treated was higher than in the untreated setup, though the soil concentration was high, which is the reverse characteristic of the above two metals (As and Cr). In Cu, 14.79 ppm was followed by 11.65 and 9.34 ppm for 10, 25, and 50%, which is higher than the amendments of untreated setup except for 50% (14.61 ppm). EK helps to excite Cu somewhat, as treated soil values were higher than the untreated soil. High copper levels have been demonstrated to harm mycorrhizal relationships and lower microbial activities (Georgieva *et al*., 2002). For Ni, values enhanced from 1.60 to 6.28 ppm for 10%, 1.60 to 6.197 for 25%, and 1.85 to 2.34 ppm for 50% from untreated to treated setup, respectively tough the absorption of 50% was treated was less in comparison to the 25%. Ni enhanced from 42.195 to 83.77 ppm for the soil, 44.78 to 89.33 ppm, and 51.68 to 110.76 ppm from 10, 25 and 50%, respectively. Microbes and soil invertebrates may be adversely affected by exposure to high Ni concentrations in soils (Vischetti *et al*., 2022). Pb has no role in biological function; instead, it has a higher toxic impact, which interrupts the enzymatic activities, seed germination, and growth of plants, but in this experiment (Zulfiqar *et al.,*2019),

Spinach absorbed Pb efficiently without harming its biomass for both untreated and treated setups. Pb concentration increased from untreated plant-78.22 to treated plant-82.69 ppm for 10%, 93.17 to 96.68 ppm for 25 %, and 88.17 to 99.54 ppm for 50%. In the case of soil, 10% of treated soil showed the highest values (92.86 ppm) over 25 and 50%. Absorption of Zn was highest among the six metals. Zn from the 10% amends responded well as ppm elevated from 163.52 to 325.07, whereas for 25% and 50%, it rose from untreated plant-106.90 to treated plant-115.51 and 131.90 to 168.50 ppm. In the case of soil, 50% increased from 349.89 to 416.9 ppm.

The EK effect made all the metals available to great extents; out of that, the high toxicity of As and Cr affected the plant absorption, whereas other metals responded well. Mao *et al*. (2015) did the same experiment with Pd, As, and Cs-contaminated paddy soil with Indian mustard, Cabbage, and Spinach to check the removal efficiency through the coupling method of EK-Phyto remediation. They found that water solubility had increased after EK treatment and that the mustard and

Table 2. Phytoaccumulation values of different metals in untreated and treated setups with *Spinach oleracea*

Metals	Amends (%)	Untreated (ppm)			Treated (ppm)		
		Soil	Plant	BAF	Soil	Plant	BAF
Cr	0	129.48	22.52	0.17	129.48	22.52	0.17
	10	89.92	38.53	0.43	147.62	33.57	0.23
	25	107.745	39.03	0.36	140.935	30.906	0.22
	50	125.665	58.53	0.47	200.57	28.595	0.14
Pb	0	62.325	96.53	1.55	62.325	96.53	1.55
	10	85.03	78.22	0.92	92.86	82.69	0.89
	25	61.27	93.17	1.52	73.975	96.68	1.31
	50	67.86	88.17	1.30	77.095	99.54	1.11
As	0	6.59	0.42	0.06	6.59	0.42	0.06
	10	7.995	0.38	0.05	7.655	0.35	0.04
	25	7.58	0.49	0.06	7.165	0.29	0.04
	50	7.10	0.39	0.05	8.12	0.34	0.04
Cu	0	53.57	7.99	0.15	53.57	7.99	0.15
	10	54.23	8.15	0.15	76.59	14.79	0.19
	25	48.87	9.11	0.19	97.06	11.65	0.12
	50	96.28	14.61	0.15	117.16	9.34	0.08
Ni	0	59.515	2.01	0.03	59.515	2.01	0.03
	10	42.195	1.60	0.04	83.77	6.28	0.07
	25	44.78	1.60	0.04	89.33	6.20	0.07
	50	51.685	1.85	0.04	110.76	2.34	0.02
Zn	0	79.485	39.90	0.50	79.485	39.90	0.50
	10	106.49	163.52	1.54	165.44	325.07	1.96
	25	151.245	106.90	0.71	120.075	115.51	0.96
	50	349.89	131.90	0.38	416.9	168.05	0.40

spinach enhanced the bioaccumulation of Pb and As. These comparable outcomes were observed by Gautam and Agrawal (2017) and Handique and Handique, (2009) using an aromatic crop (lemongrass) as a hyperaccumulator in the soil amended with SS, and absorption of Hg, Pd, and Cd was accelerated. Rathika *et al.* (2021) used biochar and EDTA to sequestrate the lead-contaminated soil, and they found chelators helped to provoke *B. juncea* for accumulation of Pb. An oligomer enzyme called phytochelatin (PCs) is produced by phytochelatin synthase, which is highly expressed in a hyperaccumulator and helps to accumulate toxins without affecting its toxicity to the plant. Generally, PCs bind with HMs and detoxify in the cellular vacuole of the plant cell (Chaudhary *et al.,*2018). Farraji *et al.,*(2014) observed that Spinach potentially accumulated Pb, and the shoot part was reported more accumulation than the root. Anova analysis applied between treated soil and the plant was highly significant as the F value is 5.07 (0.05 level). For untreated setup, F is 4.55 (0.05 level), which just satisfied the critical value (4.05), and this highly depends on the metal's availability to the plant as some metals had a poor response to the plant for metal uptake.

Impact of sewage sludge on biomass and Stress tolerance index of *Spinacia oleracea*

Total biomass and biomass per plant were estimated, and values are displayed in Fig. 3. The total biomass of the treated plant was much higher than that of the untreated plant, which denotes spinach's sustainability in bioavailable HMs in SS media. Values increased gradually, such as 6.65, 7.07, and 7.86 grams for 10, 25, and 50%; on the other hand, the total biomass of untreated plants was slow and low, ranging from lower to higher concentrations. For biomass/plant, initial weights of 10 and 25 % of both sets were increased, but in 50%, it was reduced. Values decreased from 0.74 and 0.88 to 0.56 and 0.7 grams for untreated and treated plants, respectively. This indicates that the plants suffer to survive in the higher toxicity.

Singh *et al.* (2020) reported that *Cymbopogon martinii* also enhanced its biomass in different concentrations of SS. A series of experimental articles, such as Arlo *et al.* (2022) ; Urbaniak *et al.* (2017) ; Bozkurt and Yarılgaç (2010), assured that biomass production increased in sorghum, apple tree, willow, Oak, Norway spruce, and Scots pine after adding SS into the soil. Inhabitation and reduction of biomass during growth are general profiles for advanced plants to counter the toxicity of metals (Malik *et al.,*2022).

The STI showed more impressive results for treated plants than the untreated plants, as a notable difference can be observed (Table-1). Untreated plant values such as 119.78, 134.79, and 102.19 for 10, 25, and 50%, however, compared to treated, 154.94, 160.43, and 127.83 for 10, 25, and 50%. In the case of 50%, the treated value was higher, possibly due to the higher OC after the EK effect that supported the growth of plants in higher toxic concentrations.

Bioaccumulation factor (BAF) of *Spinacia oleracea*

BAF helps to classify the plants into hyperaccumulators and non-hyper accumulator based on their metal absorption ability. If the absorption concentration exceeds 1mg kg^{-1}, it is considered a good hyperaccumulator (Cluis, 2004). It widely depends on factors such as pH, species, particle size, texture, and availability of metals (McGrath and Zhao, 2003; Al-Qahtani, 2012; Arthur *et al.,*2005), and all values are given in Table 2.

The same trend was also found in As due to the less absorption in the treated plant. A constant value (0.04)

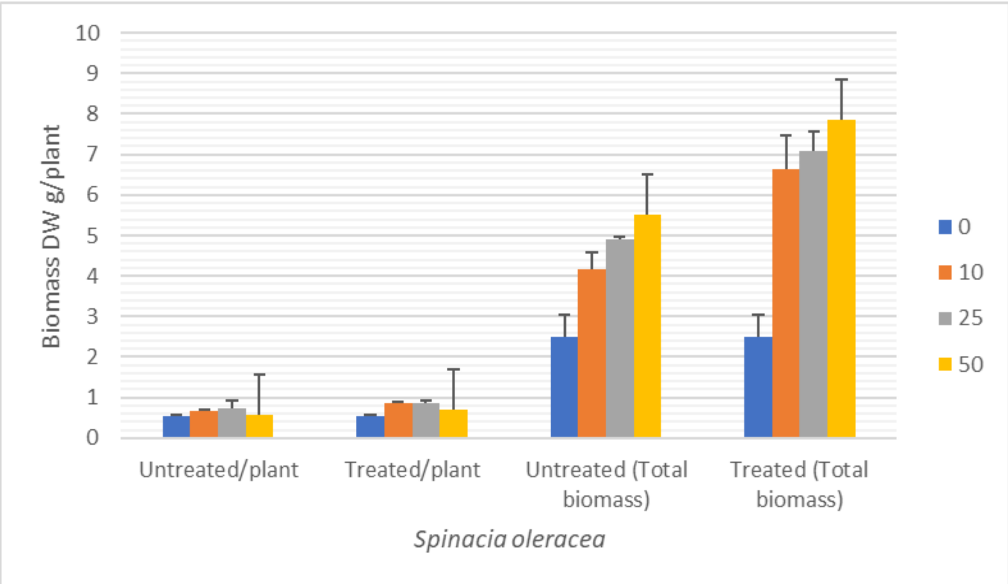

Fig. 3. *Biomass of untreated and treated sewage sludge with Spinacia oleracea at different concentrations*

was maintained in all the concentrations; however, untreated values were ununiformed as 10% showed 0.05, followed by 25% (0.06) and 50% (0.05). For Cu, 10% reported higher uptake for treated (0.19) compared to untreated. However, absorption capacity was reduced by 25% and 50%, as values are lower than the untreated setup. In the case of Ni, a constant value of BAF has been maintained for all the concentrations (0.04) for untreated setup, but a change is there for treated setup. 0.07 was seen for the initial two concentrations, while for 50%, it decreased to 0.02 as sequestration was decreased in high toxicity. BAF of Pb and Zn were highest as some concentrations were >1 in both setups. Untreated Pb values were higher than those treated, but 25 and 50% values in both setups are >1. 0.92 and 0.89 represent 10%, whereas 1.52 and 1.31 represent 25%, followed by 1.30 and 1.11 for 50% untreated and treated, respectively. 10% of Zn for both setups was >1. Values were raised from untreated-1.54 to treated-1.96, 0.71 to 0.96, and 0.38 to 0.40 for 10, 25, and 50%, respectively.

Zn is a crucial plant micronutrient (Rizwan *et al.,*2018). Hence high uptake of Zn by plants might have been involved in metabolic activities but excess absorption of Zn worsens the chloroplast and chlorophyll synthesis and inhibits plant growth and photo assimilation from leaves to root. On the other hand, nonessential metals accumulate in different parts of the plant. Guo *et al* (2019) tested Brassica with EDTA and organic acid for phytoextraction of Cd and Zn and did a comparison study with the control one. The translocation factor and bio co-efferent factor showed notable results compared to the untreated plants as treated plants did a good accumulation of Zn and Cd under the bioavailability of metals. Ugulu *et al*(2020) reported that the BAF of Spinach was < 1 for Zn, Cu, Cr, and Cd in a sample irrigated with sugar mill water whereas Zn and Mn were high in a sample irrigated with groundwater. This means these metals are easily available in edible parts (Ahmad *et al.,*2018). In post-harvesting season, metals can be claimed from plants through the Phyto-mining process can boost the finances or biomass can be used as bioenergy (Kikis *et al*., 2024).

Conclusion

EKT manipulates the metal configuration that stimulates the root to absorb beyond the permissible limit. pH, EC, and OC increased after the EKT, indicating the availability of ions with high organic matter that promote and nourish spinach to grow in a harsh/ toxic medium, improving total biomass in the treated setup. A gradual upgradation of STIs has been reported, and test species have adopted the environment without negotiating its in and outgrowth. Plants failed to sequestrate As and Cr and reported less absorption after EKT, whereas Pb and Zn were absorbed most, followed by other metals. Since edible plants act as hyperaccumulators and have a substantial risk of food chain contamination, extreme attention is needed throughout this treatment.

Conflict of Interest

The authors declare that they have no conflict of interest.

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