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Valorizing the reuse of treated municipal wastewater for Paulownia seedlings cultivation by application of Moringa waste

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Abstract

A field experiment was conducted to study the effect of treated municipal wastewater and adding defatted seeds of *Moringa oleifera* to the soil with different levels compared to tap water on the vegetative growth and chemical composition of Paulownia hybrid seedlings as well as soil chemical properties. This experiment was carried out in the experimental area of the National Research Centre, Cairo, Egypt from March to December 2019 and 2020 for three irrigation periods (3, 6 and 9 months). The use of treated municipal wastewater individually or in combination with defatted seeds of *Moringa oleifera* significantly increased all the studied growth parameters comparing to tap water (the control) in all growth intervals. The treatment of treated municipal wastewater with 10 g of defatted seeds of *Moringa oleifera* added to the soil was the best than other treatments in improving the most growth parameters. Maximal accumulation of heavy metals occurred in the roots followed by leaf and stem, respectively. A notable increase in antioxidant enzyme activity was noticed in Paulownia seedlings that were treated by treated municipal wastewater alone or with using 20 gm of defatted seeds of *Moringa oleifera*. The trees of Paulownia were found to be a promising species for trace metal tolerance in polluted soils and using treated municipal wastewater for irrigation. Remediation of soil with defatted seeds of *Moringa oleifera* had promotion effects on reducing the uptake of metal and decreasing its availability.

Key words: Heavy metals, municipal Wastewater, Paulownia seedlings, *Moringa oleifera* seeds

1. Introduction

Reuse of treated, diluted or even raw municipal wastewater in irrigation is common in most of the developing countries **[1]**. Water scarceness is not only the driver for reuse of wastewater but also the preservation of available water resources. Nutrient recovery is also an important reason for wastewater reuse and reducing their harmful environmental impact; acidification and eutrophication **[2]**. Wastewater irrigation ensures the recycling and reuse of water resources in arid lands such as Egypt and works as the economic treatment of urban sewage. Success in using treated wastewater for forest production will mostly depend on strategies that aim to optimize the quality and yield of wood, while preserving soil productivity and protecting the environment **[3]**. The direct application of wastewater on agricultural land is restricted by the extent of contamination with toxic organic, heavy metals, pathogens and chemicals **[1]**. Now, Egypt is testifying a wide range of new projects of wastewater reuse for expanding the green stretch in the desert through forestation which yields timber trees of high economic value. Usage of wastewater for forest irrigation is recommended for fuel and timber production. It is a method that helps to overcome health hazards and safe disposal of wastewater instead of its discharge into the sea **[4]**. Heavy metals are recognized as long-term hazardous contaminants that can significantly affect the aquatic life. Therefore, the contamination of wastewater with metals can limit its application in irrigation. Although heavy metals in (TMWW) effluents were found to be low and within recommended standard limits for irrigation water, many studies reported that soils were polluted with metals with the long- term irrigation.

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The accumulation of heavy metal in the soil is expected to enlarge by increasing the world water demand for agriculture. Therefore, there is a need to control the metal mobility in the receiving soils to prevent their transfer into different parts of the plant.

All naturalist products are usually safe, stakeholders tended to avail alternate organic materials for adsorptive removal of heavy metals from wastewater such as agricultural wastes which are characterized for being hugely producible and relatively cheap with their capability as bio sorbents of metal ions **[5]**. Also, they have advantages as being biodegradable, non-risky in their effects on environment and health **[6]**. Moringa was the tree of choice, it often works as a bio-remediator **[7,8]**. Seeds were used for several aims like water purification, medicine, and food **[9]**. The residue originated from oil extraction (seed-cake) was used as biofertilizers **[10]**. The seed by-product, which contains all active components, has a cumulative effect and is used to remove contaminants from aqueous solutions **[11]**.

Paulownia trees belong to family Paulowniaceae (Scrophulariaceae), which characterized by a fast-growing and short-rotation woody crop. They are deciduous hardwood trees, its leaves arranged in opposite pairs on the stem. It cultivates under several weather conditions and in a wide variety of soil types even poor ones **[12]**. Paulownia wood is used as a good material for composting, pulp paper, lumber, coal and firewood. Also appropriate for making musical instruments, boxes, light weight skis, chests and furniture making **[13]**. Paulownia is an ornamental tree, now it is widely cultivated for the protection of environmental pollution, increase soil texture, reduce soil erosion, and improve soil fertility by adding nitrogen to the soil **[14]**.

Under the conditions of overpopulation and to mitigate the water stress, the reuse of wastewater must be applied. Therefore, this work was conducted to assess the potential of reusing treated municipal wastewater for irrigation of Paulownia hybrid seedlings (*P. elongate* ×*P. fortunei*), as well as the effect of DSMO on heavy metals accumulation in the soil.

2. Experimental

Homogenous seedlings of Paulownia hybrid (*P. elongata x P. fortunei*) one-year-old averaged 25 cm in height were used as plant material. The seedlings were obtained from the Nursery of Forestry Department, Horticulture Research Institute, Agriculture Research Centre, Giza Egypt. This study was carried out in the experimental area of the National Research Centre, Cairo, Egypt. The study lasted for 9 months from March to December 2019 and 2020 to investigate the effects of irrigation with treated municipal wastewater (TMWW) and the usage of defatted seeds of *Moringa oleifera* (DSMO) on the vegetative growth and chemical composition of Paulownia hybrid and soil chemical properties. The seedlings were planted on 1st February 2019 in plastic pots (30 cm diameter and 25 cm height, with holes in the bottom) filled with a mixture from clay and sand 1:1v/v (one seedling/pot). All seedlings were irrigated with tap water for one month until adaptation on 1st March 2019 after that the treatments have been started with treated municipal wastewater. Defatted seeds of *Moringa oleifera* were added to the soil every month and a half starting on 1st March with different additives (10 and 20 g / pot).

Treated municipal wastewater TMWW was obtained from a nearby wastewater treatment plant that receives urban wastewater for the irrigation of plants. Screening, de-gritting, primary settling, aeration, secondary settling, and disinfection were all part of the treatment process. Table 1 shows an overview of the water used for irrigation according to the standard methods for the examination of water and wastewater, **[15]**.

Phytochemical constituents of defatted seeds of *Moringa oleifera* were estimated in this study such as; the polysaccharide yield was extracted by Gaafar *et al*., **[16]** method, the total carbohydrate was determined by Dubois *et al*., **[17]** analyses and Total phenol contents were determined according to Singleton *et al*., **[18]**. The polysaccharide yield of defatted seeds of *Moringa oleifera* was 23.5±0.02 % and total phenol content was 16.05 ± 0.10 mg/g and total carbohydrate was 80%.

2.1. The experiments design

A complete randomized design was used for the experiment. The four treatments (1: Tap water for control, 2: TMWW, 3: TMWW + 10 g/ pot DSMO, 4: TMWW + 20 g/ pot DSMO) were replicated three times, and each repetition contained four seedlings. The means among all used treatments were compared by Duncan's Multiple Range Test, according to Snedecor and Cochran **[19]**. At the end of each period (3, 6 and 9 months), three seedlings for each treatment were chosen randomly to determine the following parameters:

Table 1. The average composition of the water used in experiment irrigation.

parameters	Unit	TMWW	Tap water	Permissible Limits for reuse Egyptian code no. 501
PH	-	6.9	6.79	-
Total Suspended Solids	mg/l	21	ND	300
Total Dissolved Solids	mg/l	614	34	2000
Turbidity	NTU	161	ND	-
Chemical Oxygen Demand	mgO_2/l	340	ND	-
Biological Oxygen Demand	mgO_2/l	147	ND	350
Phenol	mg/l	ND	ND	0.002
Total phosphate	$mgPO_4/l$	5.7	ND	30
SAR	-	1.1	ND	6-9
Sodium	mg Na/l	189	52	230
Sulfate	$mgSO_4/l$	311	ND	500
E. Coli	CFU/100ml	1242	ND	-
Chromium	mg/L	<0.001	<0.001	0.1
Cadmium	mg/L	<0.001	<0.001	0.01
Lead	mg/L	0.18	<0.001	5.0
Copper	mg/L	<0.01	<0.01	0.2
Iron	mg/L	0.8	<0.01	5.0
Manganese	mg/L	0.32	<0.01	0.2
Nickel	mg/L	<0.001	<0.001	0.2
Zinc	mg/L	<0.01	<0.01	5.0

N.D: Not Detected, [treated municipal wastewater (TMWW)]

2.1.1. Vegetative growth parameters

Plant height (cm), leaves number/ plant, the diameter of stem and root (cm), root length (cm) and fresh and dry weight of stems and roots (g)/ plant.

2.1.2. Chemical constitution of the different plant parts (leaves, shoots and roots)

Metal ion concentrations (Pb, Fe, Mn) in all samples were measured using the Agilent 5100 Synchronous Vertical Dual View (SVDV) ICP-OES according to APHA **[15]**. All samples were digested to have acceptable matrix for measuring using Anton-Paar microwave digestion system (Multiwave PRO).

2.2. Antioxidant enzymes extraction

The antioxidant enzymes were extracted and determined at the end of the experimental period, where 0.1g of fresh plant material was homogenized in 5 ml of ice-cold buffer phosphate (pH 7.4). The homogenate was centrifuged at 10,000 rpm for 30 min and supernatant was collected. The resulting supernatant was used for determination of enzyme activities. Superoxide dismutase enzyme activity was measured with spectrophotometer according to Marklund and Marklund method **[20]**, the activity of Catalase was estimated by method of Nakano and Asada **[21]**. The reduced glutathione activity in supernatant was determined by method of Beutler and Kelly **[22]**.

2.3 Analysis of soil sample

At the end of the experiment, heavy metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) were extracted from soil by DTPA (di-ethylene tri-amine penta-acetic acid) and measured in the solution by ICP-OES **[23]**.

3. Results and discussion

3.1. The Vegetative growth

Results of the vegetative growth parameters (plant height, stem diameter, stem fresh and dry weight and leaves number) of paulownia seedlings are shown in Table 2. The results indicated that the application of treated municipal wastewater individually or with the application of DSMO treatments significantly increased all the studied vegetative growth parameters compared with tap water (the control) in all growth intervals. Generally, the highest values in all previous parameters were recorded with treated municipal wastewater plus 10 g of DSMO treatment during all periods except plant height and stem fresh weight were significantly higher after 6 months with using treated municipal wastewater alone compared with other treatments. The results indicated that the vegetative growth parameters at any treatments increased gradually with the irrigation period.

Table 2. Effect of treated municipal wastewater and defatted seeds of *Moringa oleifera* on vegetative growth parameters of paulownia hybrid (*P. elongate*× *P. fortunei*) seedlings

Treatments	3 months	6 months	9 months	3 months	6 months	9 months
	Plant height (cm)			Stem diameter (cm)		
Tap water	53.0d	70.0d	81.0c	0.75c	0.78c	1.00c
TMWW	80.3b	106.8a	113.3a	0.87b	1.10ab	1.23b
TMWW + 10 gm DSMO	90.0a	96.0b	109.7a	1.00a	1.17a	1.70a
TMWW + 20 gm DSMO	68.0c	81.7c	93.0b	0.81bc	1.00b	1.10bc
	Stem fresh weight (g)			Stem dry weight (g)		
Tap water	11.3d	17.0d	30.0c	5.7c	9.3c	15.9d
TMWW	23.2b	31.0a	45.2b	12.3a	16.9a	24.4b
TMWW + 10 gm DSMO	27.7a	29.3b	64.7a	14.7a	16.0a	36.3a
TMWW + 20 gm DSMO	21.5c	23.3c	33.5c	9.8b	12.6b	18.4c
	Leaves number/plant					
Tap water	11.3c	13.7bc	12.0bc			
TMWW	15.0b	14.0b	13.0b			
TMWW + 10 gm DSMO	18.0a	16.3a	14.3a			
TMWW + 20 gm DSMO	11.7c	12.3c	11.3c			

Similar letters within a column are not significantly different at the 0.05 level probabilities by Duncan's Multiple Range Test. [Treated municipal wastewater (TMWW), defatted seeds of *Moringa oleifera* (DSMO)]

3.2. Root characters

The presented data of Table 3 showed that the application of treated municipal wastewater did not show any negative effect on the root characters while it improved these characters compared to tap water. The highest values of root diameter, root length, roots fresh and dry weights were obtained when using treated municipal wastewater with10 g of DSMO treatment compared to other treatments. The tap water presented a significant decrease in values of root characters. This trend was observed in the three studied growth periods.

The results in Tables (2 and 3) were noted by many investigators who decided that wastewater had a promoting effect on the vegetative growth of trees due to providing nutrients and organic matter in the soil and improving its physical characteristic, which reflected on the growth by promoting cell elongation and division (Guo and Sims, on *Eucalyptus globulus* **[24]**, Singh and Bhati, on *Dalbergia sissoo* **[25]**, Abdel Aziz *et al.*, on *Swietenia mahagoni* **[26]** and Hashish *et al.*, on some woody trees **[27]**). Also, this positive effect of defatted seeds of *Moringa oleifera* (DSMO) might be attributed to its formation from cellulose, hemicellulose and lignin. These functional groups consist of macromolecules that have the ability to absorb metal ions through complexation or ion exchange **[28]** or their richness in vitamins, minerals and proteins, where seed-cake was used as biofertilizers **[10,29]**.

Table 3. Effect of treated municipal wastewater and defatted seeds of *Moringa oleifera* on root characters of paulownia hybrid (*P. elongate*× *P. fortunei*) seedlings

Treatments	3 months	6 months	9 months	3 months	6 months	9 months
	Root diameter (cm)			Root length (cm)		
Tap water	1.00b	1.03c	1.20c	30.0d	47.0d	45.3d
TMWW	1.10b	1.47a	1.60b	50.0b	53.3b	59.0b
TMWW + 10 gm DSMO	1.40a	1.57a	2.20a	54.0a	59.3a	66.3a
TMWW + 20 gm DSMO	1.20ab	1.27b	1.40b	45.0c	49.8c	54.0c
	Root fresh weight (g)			Root dry weight (g)		
Tap water	25.0d	31.8d	37.7d	8.8c	11.5c	13.6d
TMWW	30.2c	44.2b	56.0b	10.9b	16.5a	20.6b
TMWW + 10 gm DSMO	35.3a	47.2a	80.3a	12.5a	17.7a	26.4a
TMWW + 20 gm DSMO	33.2b	36.0c	50.7c	12.2ab	13.0b	17.7c

Similar letters within a column are not significantly different at the 0.05 level probabilities by Duncan's Multiple Range Test. [Treated municipal wastewater (TMWW), defatted seeds of *Moringa oleifera* (DSMO)]

3.2. Chemical composition

It is well known that metals are absorbed and trans-located by plants with differing degrees. Although that the wastewater contains low levels of heavy metals, the plant samples revealed distinct metal values within its parts incomparable with the plant irrigated with tap water (Control). Generally, irrigation with TMWW effluent gave the highest concentrations of Mn, Pb and Fe in roots, stems and leaves of Paulownia plants, Table 4.

The increase of Mn, Pb and Fe ions concentrations in different parts of plant might be attributed to increasing the residence of root zone by applying TMWW for irrigation that reflected on their uptake by roots. The highest accumulated levels of all studied metals were noticed in roots comparing to the other parts of Paulownia plant. The results agree with the findings of Klink, **[30]** who found that concentrations of heavy metals (Fe, Mn, Zn, Cu, Cd, Pb, Ni, Co and Cr) tended to accumulate in root more than that in leaves and shoots. With the continuous irrigation of Paulownia plants with TMWW, more accumulation of metals was observed due to the progressive increase of vegetative growth. The findings are consistent with those of EL-Sayed **[31]** who showed that irrigation with secondary treated wastewater was increased the levels of Fe, Mn, Pb, and Ni in leaves, stems and roots of trees (*Acacia stenophylla*, *Acacia saligna* and *Ceratonia siliqua*) compared with tap water. Also, Chaoua, *et al*., **[32]** found that metals present in irrigation wastewater appear to accumulate in soils, where they have the ability to become bioavailable for crops, as compared to tap water.

For all metal ions examined, the increase in quantities of metal ions within the whole plant as a result of irrigation with TMWW effluent compared to tap water after 9 months ranged from 2 to 7 times, and can be arranged in theis order. Mn>Pb> Fe.

Amendment of soil with DSMO significantly increases the binding ability of metal within the remediated soil and decreasing the availability of metals uptake by Paulownia plants. This uptake not only results in decreasing heavy metal accumulation in plant tissue but also definitely affects the growth of plants. The reduction in metal ions availability might be attributed to the presences of polar functional groups of lignin and tannin compounds in DSMO, which have methoxyl, hydroxyl-aliphatic, carboxyl, and amino groups. These groups have the strong ability to bind metal ions by chelation to form stable complexes. The results agree with Park *et al*.,**[33]** who mentions that presence of insoluble organic material in soil, containing high molecular humic acids, encourages the binding of metals and limits their immobilization.

3.3. Soil remediation

Phytostabilization of metal ions within the soil irrigated with TMWW was performed by adding different amounts of DSMO. Table 5 showed the DTPA-extractable Cu, Cr, Cd, Ni, Pb, Fe, Mn and Zn after 9 months of irrigation. The results showed that in general the highest values were observed in the case of irrigation with TMWW; these results might be because the heavy metals accumulated in soil from the continuous irrigation of soil with municipal wastewater. Similar results have been reported by Ali *et al.*, **[34]** who mentioned that extractable metals increased as irrigation period increased by sewage effluent. Utilization of DSMO as an amendment could limit the migration of metal contaminants from wastewater to the soil as well as their accumulation in living plants by the mechanism of phytostabilization. The results showed that increasing the amount of DSMO to 20 g/pot was more effective in immobilizing metal ions within the soil. The results agree to a great extent with Hassanein *et al*., **[35]** that mixed coagulant defatted *Moringa oleifera* seeds with the soil and examine the stress of cadmium ions on *Triticum aestivum* plants, they found that the application of Moringa seeds powder can detoxify the toxic effect of cadmium as it can coagulate Cd^{2+} ions from the soil by the presence of proteins having coagulation properties.

Table 4. Effect of treated municipal wastewater and defatted seeds of *Moringa oleifera* on the levels of Pb, Fe and Mn, in roots (R), stems (S) and leaves (L)

Treatments	Periods								
	3 months			6 months			9 months		
	Root	Stem	Leave	Root	Stem	Leave	Root	Stem	Leave
Pb (mg/kg)									
Tap water	6.3^{b}	0.2^{b}	1.5^{c}	7.3^{b}	0.25^{b}	2.5^{c}	7.5^{b}	0.25^{b}	2.6^{c}
TMWW	20.9^{a}	0.35^{a}	4.3^{a}	35.8^{a}	0.5^{a}	6.5^{b}	37.8^{a}	0.5^{a}	5.8^{b}
TMWW + 10 gm DSMO	5.5^{c}	0.01^{c}	3.5^{b}	5.36^{c}	0.01^{c}	5.5^{a}	5.9^{b}	0.01^{c}	4.8^{a}
TMWW + 20 gm DSMO	4.84^{d}	0.01^{c}	3.1^{b}	5.12^{c}	0.01^{c}	4.25^{b}	5.9^{b}	0.01^{c}	4.9^{b}
Fe (mg/kg)									
Tap water	547.63^{c}	50.0^{b}	106.5^{c}	839^{d}	72.56^{b}	371.6^{c}	939^{c}	79.56^{c}	398^{d}
TMWW	1527.3^{a}	78.9^{a}	455^{a}	2872^{a}	115.7^{a}	1022.7^{a}	3872^{a}	115.7^{a}	1422.7^{a}
TMWW + 10 gm DSMO	637^{b}	46.2^{c}	121.5^{c}	1157^{b}	66.74^{c}	506.5^{b}	1457^{b}	95.3_{b}	906.5^{b}
TMWW + 20 gm DSMO	602.6^{b}	41.9^{d}	180^{b}	910^{c}	42.98^{d}	494^{b}	980^{c}	42.98^{d}	694^{c}
Mn (mg/kg)									
Tap water	19.03^{b}	6.7^{b}	9.7^{b}	40.01^{c}	6.42^{b}	14.3^{c}	43.2^{c}	6.7^{c}	14.5^{d}

TMWW	48.8^{a}	10.6^{a}	18.7^{a}	102.2^{a}	12.6^{a}	53.3^{a}	285.3^{a}	16.16^{a}	89.3^{a}
TMWW + 10 gm DSMO	17.73^{bc}	6.19^{c}	9.4^{b}	55.2^{b}	7.97^{b}	21.6^{b}	65.2^{b}	8.12^{b}	35.6^{b}
TMWW + 20 gm DSMO	16.3^{c}	5.97^{c}	9.5^{b}	40.1^{c}	6.45^{b}	14.3^{c}	49.01^{c}	8.6^{b}	28.3^{c}

Treated municipal wastewater (TMWW), defatted seeds of *Moringa oleifera* (DSMO)

Table 5. The DTPA-extractable-heavy metals concentration from the soil used in agricultural of Paulownia seedlings after 9 months of irrigation with municipal wastewater.

treatments	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
					mg/l			
Tap water	ND	0.4	0.25	2.5	1.3	0.75	1.28	3.5
(TMWW)	0.05	1.5	0.75	3.5	2.4	1.90	3.25	7.15
(TMWW) + 10 gm (DSMO)	0.03	0.3	0.45	2.4	1.1	0.75	0.87	4.3
(TMWW) + 20 gm (DSMO)	0.02	0.17	0.38	2.0	0.87	0.38	0.85	3.9

Treated municipal wastewater (TMWW), defatted seeds of *Moringa oleifera* (DSMO)

3.4. **Antioxidant enzymes activities of paulownia seedlings**

The high activity of catalase and superoxide dismutase (SOD) enzymes was found for treatment with treated municipal wastewater alone (100.26 and 22.34 U/mg tissue, respectively) and treatment with treated municipal wastewater plus 20 g of DSMO (99.72 and 21.69 U/mg tissue, respectively) compared to using tap water (89.04 and 19.50 U/mg) or using 10 g of DSMO with treated municipal wastewater treatment (88.95 and 19.06 U/mg) as shown in Table 6. While, the activity of Glutathione peroxidase was stimulated markedly only in treated municipal wastewater treatment (210.80 U/mg) compared with other treatments.

By presenting the results Heavy metal toxicity has a variety of effects on plants at the cellular and molecular levels. It interferes with physiological and biochemical processes such as photosynthesis, gas exchange, respiration, and enzyme denaturation, as well as blocking functional groups of metabolically important molecules, hormonal balance, and protein synthesis **[36-38]**. Heavy metals such as Cu, Cd, Fe, and Zn can be formed ROS (reactive oxygen species) active **[39]**. Antioxidant enzymes protect cells from damage caused by exposure to certain highly reactive species like ROS **[40]**. Wang *et al*., **[41]** found that *P. fortune* has a high tolerance for Pb metal. This is due to the increased activity of some enzyme, including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) and glutathione peroxidase (GPX)) activities. It is clear that the role of DSMO in absorbing the heavy metals and limiting the increase in antioxidant enzymes caused by TMWW irrigation.

Table 6. Effect of treated municipal wastewater and defatted seeds of *Moringa oleifera* on some antioxidant enzymes activity U/ mg tissue of paulownia hybrid (*P. elongate, P. fortunei*) seedlings.

Treatments	Antioxidant enzyme activity U/ mg tissue		
	catalase	Superoxide dismutase	Glutathione peroxidase
potable water	89.04^{b}	19.50^{b}	198.55^{b}
(TMWW)	100.26^{a}	22.34^{a}	210.80^{a}
(TMWW) + 10 gm (DSMO)	88.95^{b}	19.06^{b}	199.75^{b}
(TMWW) + 20 gm (DSMO)	99.72^{a}	21.69^{a}	199.71^{b}

Similar letters within a column are not significantly different at the 0.05 level probabilities by Duncan's Multiple Range Test.
Treated municipal wastewater (TMWW), defatted seeds of *Moringa oleifera* (DSMO).

4. Conclusion

Utilization of treated wastewater is an obligatory stage to preserve the environmental resources and to fulfill the gap between water demand and supply. Irrigation of woody plants such as Paulownia hybrid trees can be considered as a promising approach in reusing treated municipal wastewater. Amendment of metal contaminated soils with defatted seeds of *Moringa oleifera* could limit the migration of metal contamination from wastewater to the soil as well as their accumulation in living plants by the mechanism of phytostabilization. Furthermore, the use of treated municipal wastewater with 10 g of defatted seeds of *Moringa oleifera* was the best than other treatments in improving the most growth parameters.

5. Conflicts of interest

"There are no conflicts to declare".

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