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Assessment of genotoxic effects of wastewater of Kitchener pool, Nile Delta Region, North Egypt, using *Allium* test

Aziza S. El-Kholy*, Soliman A. Haroun and May Labeeb

Abstract

Background In the present study, *Allium* test bioassay was utilized to evaluate the effects of mixed wastewater of agricultural and sewage effluents at Kitchener pool, Gharbia governate, Middle Delta region, North Egypt. Germination indices, mitotic index and aberrations, α, β-esterase isozymes and inter-simple sequence repeat (ISSR) fingerprinting were tested by different concentrations of the wastewater (tap water as control, 25%, 50% and 100% wastewater).

Results Water analysis recorded high levels of electrical conductivity, cations and anions compared to control, but were in the permitted limits according to FAO (Food and Agricultural Organization) except Mg^{2+} and K^{1+} were above the limits. P, N and heavy metals as Pb, Mn and Ni were also higher than the control. Germination indices showed reduction for all parameters studied (root and shoot lengths, root and shoot fresh and dry weights, and tolerance index). Mitotic index decreased, and the percentage of mitotic aberrations increased as the concentration of treatments increased and the time prolonged. Different types of aberrations were recorded in all treatments and its percentage is time and dose independent. Goat cells are the most common type recorded after different times in all treatments. The expression of α, β-esterase enzymes showed variation in different treatments compared to control and ISSR profiles showed considerable polymorphism. Concentration of 25% mixed water induced different profiles for expression of both α- and β-esterase from other treatments, and the cluster analysis based on polymorphism in ISSR fingerprinting revealed the distinction of plants treated with this concentration and the control plants from those treated with high concentrations.

Conclusion It was suggested that concentration of 25% mixed water may be suitable for growth and act as fertilizer. Mixed water from this pool may be genotoxic for *Allium cepa* plants at early growth if it is used for irrigation in its present form and usage of this wastewater for agricultural purposes may be harmful and must be partially treated and biologically tested before use.

Keywords *Allium cepa*, Mitotic aberrations, Germination indices, Isozymes, ISSR fingerprinting

1 Background

The biotic and abiotic elements of the environment are negatively impacted by pollution. Water resource pollution is a major environmental challenge that affects the balance of natural ecosystems because of the increased release of dangerous chemicals and pollutants into the environment [21, 33]. The excessive influx of garbage from many sources into streams, pools and rivers has increased pollution, which negatively impacts both plants and animals as well as people [45]. Heavy metals may be

*Correspondence:
Aziza S. El-Kholy
aziza.elkhouli@sci.kfs.edu.eg
Botany and Microbiology Department, Faculty of Science, Kafrelsheikh University, Kafr Elsheikh, Egypt

released into the water due to wastes of industries and are slowly wiped off from ecosystems and may accumulate in different organisms causing series diseases. Exposure to chemical agents and pollutants has negative consequences on exposed species and have an impact on subsequent generations because these modifications may be inheritable, these consequences have emerged as being concerning [15]. Several researchers and governmental organizations are interested in this worldwide issue and are investigating its direct and indirect effects on the health of living organisms [30, 45].

There is no doubt that irrigation from rivers and lakes is a major source of support for agriculture in many nations. Unfortunately, water supply is insufficient in semiarid and arid regions. As a result, wastewater used to irrigate gardens, fields and many other agricultural purposes as an easy source of water without any consideration for its consequences. People that consume plants cultivated in contaminated soils may have health issues such as mental retardation, diarrhea, liver and kidney damage [4, 51]. Pollutants not only have an immediate negative impact on health, but they can also be mutagenic or toxic, which can result in human afflictions including cancer, atherosclerosis, cardiovascular disease and early aging [41].

According to recent studies, contaminants stimulate the generation of reactive oxygen species (ROS), which causes oxidative damage. With the purpose of quenching ROS and preventing oxidative cell damage under stressful conditions, organisms activate both non-enzymatic and enzymatic defensive mechanisms [46]. A possible biomarker of heavy metal pollution is esterase enzyme variation [38]. Genotoxicity has been detected using molecular markers [7] which have been effectively used to screen the drainage water's mutagenic effects. ISSR assay was mentioned as a more repeatable procedure and became more beneficial. This tool becomes extremely crucial since screening for genotoxic effects requires comparing patterns made by untreated (control) and treated plants [27, 54]. Furthermore, ISSR markers are highly polymorphic and regarded as effective in assessing the genotoxic potential of heavy metals [10].

Plant bioassays as *Allium cepa, Vicia faba and Pisum sativum* tests have been utilized for monitoring the potential synergistic effects of pollutants including chemicals [32, 50, 51], sewage wastewater, agricultural drainage wastewater and industrial wastes [33, 34, 44] and nanoparticles [28, 29], respectively. *Allium cepa* is a great test plant model for detecting of the harmful effects of substances. In this study, *A. cepa* aberration bioassay was utilized as a short-term test and low cost-effective indicator of toxicity in monitoring of water pollution. Germination indices, mitotic index and aberrations, α, β-esterase isozymes and ISSR fingerprinting were tested by different concentrations of the wastewater.

2 Methods

2.1 Study area and water samples and analysis

Qotour district is located at Gharbia Governorate in the Nile Delta region in Egypt (Fig. 1). Water samples were collected from Kitchener pool (30° 57′ 44.903″ E, 30° 57′ 59.618″ N) from Qutour district (Fig. 2) during year 2021. Three concentrations of mixed water (25%, 50% and 100%) were prepared and applied for the experiment and tap water used as control. Water analysis was carried out at central laboratory of Kafrelsheikh University and genetic and molecular biology laboratory at faculty of science Kafrelsheikh University, Egypt. PH number was measured using 3510 PH meter (Jenwary model). Electrical conductivity was measured using 4510 conductivity meter (Jenwary model). Total nitrogen was measured using Kjeldahl apparatus (VELP model). Na^{1+}, K^{1+}, Ca^{2+}, Mg^{2+}, Mn, Cu, Zn, Co, Ni, Cd and Pb were measured using atomic absorption spectrophotometry (GBC Avanta Σ model). Cl^{1-}, HCO_3^{1-} and SO_4^{2-} were measured by titration method as in Gaagai et al. [17].

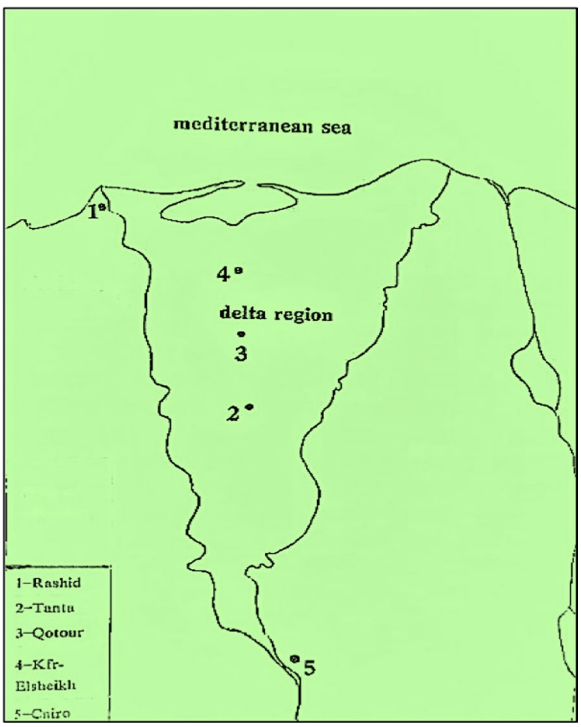

Fig. 1 Map of the Nile Delta in Egypt including study area (Qotour district)

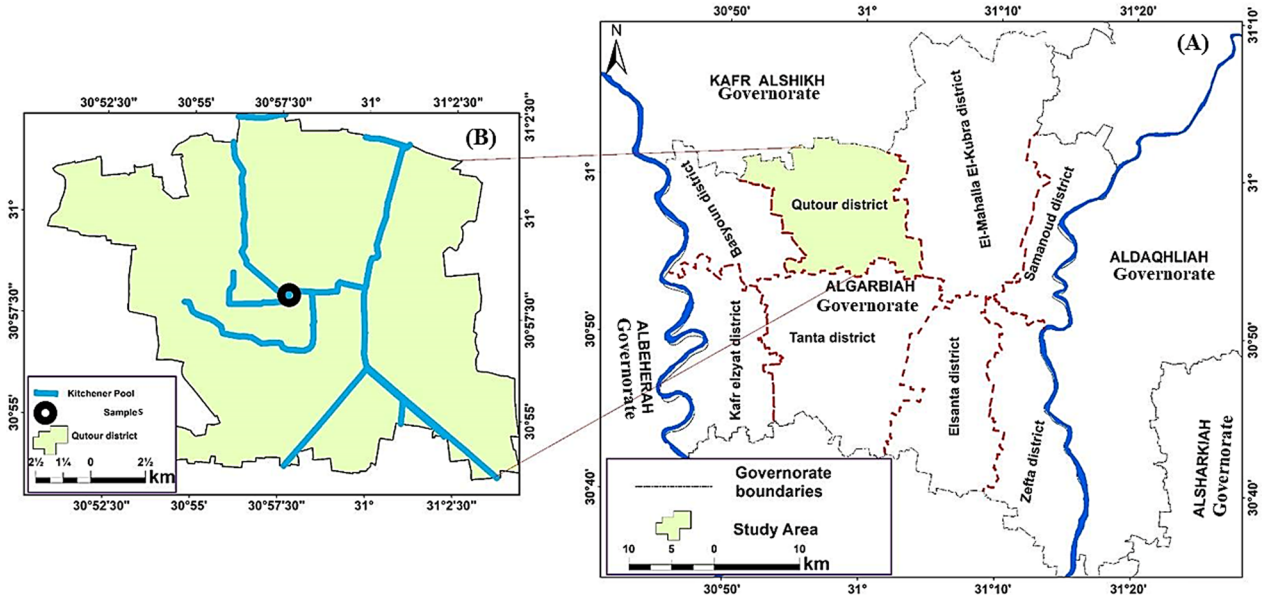

Fig. 2 **A** Map of the study area in Gharbia Governorate (Qutour district); **B** small map of the location of samples at Kitchener pool in Qutour district in the Governorate

2.2 Germination studies

Onion bulbs of medium sizes were immersed in water to the middle with each concentration (25%, 50% and 100% of mixed water) and tap water as control. Three replicates were used for each treatment as well as control. Germination indices were recorded as length, fresh and dry weights for roots and shoots at the end of 12-day period of treatment. The tolerance index of seedlings was calculated according to [52] by the following formula:

$$\text{Tolerance index} = \frac{\text{Mean length of longest root in Sample}}{\text{Mean length of longest root in control}}$$

2.3 Cytological studies

Onion bulbs were immersed in water to the middle with above concentrations and tap water as control for three periods of time 12, 24 and 36 h for cytological studies. 1–2 cm long of each root was cut and fixed in a freshly prepared Carney's fixative solution for 3 h. Fixed roots were kept in 70% ethyl alcohol in the refrigerator until use. Treated roots were hydrolyzed in 1 N HCl at 60 °C for 10 min and stained in leuco-basic fuchsin stain for 3 h at room temperature. Five well-spread slides of each treatment were examined carefully using binuclear light microscope. Mitotic indexes and mitotic abnormalities were calculated as percentage. Types and percentages of abnormalities were also recorded.

2.4 Isozyme analysis

At the end of 12-day period of germination, 500 mg of leaves for each treatment was grinded in a mortar on ice bath using 1 ml of 0.025 sodium phosphate buffer pH = 7.25 with 20% (W/V) sucrose, left for two hours in the refrigerator and mixed using vortex every 15 min. Samples were centrifuged at 16,000 rpm for 20 min at 4 °C, and supernatants were kept at − 20 °C until used. Native polyacrylamide gel electrophoresis at 10% (w/v) was utilized for separation of isozymes (α- and β-esterase), and the gels were dyed substrates for the desired enzyme according to Soltis et al. [49].

2.5 DNA analysis

At the end of 12-day period of germination, DNA was extracted from young leaves according to manufacturer protocol of GeneJET Genomic DNA Purification Kit (K0721/ Thermo fisher). Total genomic DNA was amplified through Gene Amp Polymerase Chain Reaction (PCR) system cycler through specific ISSR primers (Table 5) in 20 µl reaction volume containing 1 µl from the primer, 2 µl genomic DNA (20 ng), 10 µl Dream Taq PCR Master MIX (Thermo Fisher Scientific, Inc.) and 7 µl dd H2O. Cycling condition was conducted as follows: initial denaturation for 4 min at 94 °C, 45 cycles of denaturation at 94 °C for 1 min, 1 min for annealing at 58 °C, extension for 2 min at 72 °C and finally extension at 72 °C for 8 min. DNA was visualized using 10 µl from

PCR products on 1.5% agarose in TBE buffer with ethidium bromide at 100 V for 1 h and photographed by gel documentation system (Geldocit, UVP, England).

2.6 Data analysis

Data were statistically analyzed using one-way ANOVA (analysis of variance). DNA ISSR Bands were scored in binary matrices as 1 for presence and 0 for absence, and the similarity between different treatments was calculated using the Dice coefficient of similarity [13] using the NTSYS-pc software version 2.02 [43]. Building a distance tree to show the distances between the plants under study was done using the unweighted pair group approach using unweighted pair group method with arithmetic mean (UPGMA) [48] as implemented in the NTSYS-pc.

3 Results

In the present study, there was no difference in pH value between tap water and mixed water where the values (Table 1) were in the permitted limit according to MWE [39]. The electric conductivity value is high in mixed water recording 1.24 ds/m compared to 0.39 in control, but in the permitted limit according to FAO [6]. In relation to cations, concentrations of Mg^{2+} and K^{1+} measured in mixed water (6.33 and 0.28 meq/l) are high compared to control (2.76 and 0.16 meq/l) and FAO limits (4.94 and 0.05 meq/l), but Ca^{2+}, Na^{1+} and all anions (Cl^{1-}, SO_4^{2-} and HCO_3^{1-}) were in the permitted limits according to FAO (Table 1). The analysis of macroelements (P, N) and heavy metals also shows high values compared to control except Cu and Zn and N content is very high recording 12.53 mg/l against 2.8 mg/l in control which is probably due to sewage effluents (Table 2).

Root length significantly decreased as the concentration increased after twelve days of germination (Table 3, Fig. 3) recording significant values of 5.00 and 4.7 cm compared to the control (8.67 cm). Shoot length measurement significantly decreased in mixed water as the concentration increased compared to control recording the highest value (7.83 cm) at 25% and the lowest values at the high concentrations (50 and 100%) with values (3.44 and 2.53 cm), respectively, compared to control (13.39 cm).

Root fresh and dry weights decreased as the concentration increased (Table 3), showing significant reduction in root fresh weight for highest concentration 100% with value of 0.91 gm compared to control (1.84 gm). All concentrations showed nonsignificant reduction in root dry weight compared to control. Shoot fresh weight and shoot dry weight also decreased as the concentration of mixed water increased, showing significant reduction in shoot fresh weight for treatments of 50% and 100% of mixed water with values of (4.8 gm and 2.95 gm) compared to control (15.34 gm). Different treatments of mixed water showed highly significant low values of tolerance index (0.57, 0.53 and 0.54) for concentrations 25%, 50% and 100% of mixed wastewater, respectively.

Table 4 presents low values of MI except concentration of 25% at time 12-h treatment (12.40%) and 24 h (11.10) compared to control (11.69%) and enhancement in percentage of total abnormalities with increase in

Table 1 Water analysis of PH, EC, cations and anions in mixed wastewater and tap water (control)

Sample	Water characters								
	General character		Cations				Anions		
	PH	EC ds/m	Ca^{2+} meq/l	Mg^{2+} meq/l	Na^{1+} meq/l	K^{1+} meq/l	Cl^{1-} meq/l	SO_4^{2-} meq/l	HCO_3^{1-} meq/l
Tap water	7.35 ± 0.011	0.39 ± 0.01041	2.33 ± 0.29	2.76 ± 0.18	1.41 ± 0.26	0.16 ± 0.026	1.4 ± 0.11	2.230 ± 0.04509	2.73 ± 0.06
Mixed water	7.27 ± 0.012	1.24 ± 0.02000	2 ± 0.5	6.33 ± 0.11	6.15 ± 0.66	0.28 ± 0.026	4.87 ± 0.26	4.800 ± 0.3894	5 ± 0.4
FAO*	8.5	3	19.960	4.938	39.977	0.051	29.215	19.968	10.004

*FAO**: Food and Agricultural Organization

Table 2 Water analysis of macroelements and heavy metals in tap water (control) and mixed wastewater

Sample	Water characters							
	Macroelements		Heavy metals					
	P mg/l	N mg/l	Pb mg/l	Mn mg/l	Cu mg/l	Ni mg/l	Zn mg/l	Co and Cd
Tap water	0.16 ± 0.04	2.8 ± 0.15	0.1 ± 0.08	0.16 ± 0.006	0.09 ± 0.03	0.03 ± 0.01	1.26 ± 0.3	0.0
Mixed water	0.55 ± 0.12	12.53 ± 0.29	0.2 ± 0.06	2.34 ± 0.17	0.07 ± 0.02	0.04 ± 0.005	0.66 ± 0.4	0.0

Table 3 Measurements of root and shoot indices (root and shoot length (cm), fresh and dry weights (gm) of *Allium cepa* treated with tap water as control, 25%, 50% and 100% of mixed wastewater at 12th day after germination

Treatments	Germination indices						
	Root indices (Mean ± SE)			Shoot indices (Mean ± SE)			Tolerance index of seedling
	Root length (cm)	Root Fresh weight (gm)	Root dry weight (gm)	Shoot length (cm)	Shoot Fresh weight (gm)	Shoot dry weight (gm)	
Control	8.67 ± 0.9	1.84 ± 0.1	0.097 ± 0.006	13.39 ± 1.49	15.34 ± 2.21	1.15 ± 0.13	1
25%	5.00* ± 0.7	1.05 ± 0.1	0.0715 ± 0.01	7.83* ± 1.92	11.22 ± 4.20	1.098 ± 0.36	0.57**** ± 0.03
50%	4.7* ± 0.9	1.29 ± 0.1	0.078 ± 0.005	3.44** ± 0.72	4.8* ± 1.84	0.51 ± 0.18	0.53**** ± 0.04
100%	4.7* ± 0.7	0.91* ± 0.3	0.058 ± 0.01	2.53*** ± 0.33	2.95* ± 0.25	0.37 ± 0.02	0.54**** ± 0.03

*, **, *** and **** Significant at 0.05, 0.01, 0.001 and 0.0001 probability levels, respectively

Fig. 3 *Allium cepa* treated with **1** tap water as control, **2** 25%, **3** 50% and **4** 100% of mixed wastewater at 12th day after germination

concentration of treatments. Different types of abnormalities such as goat cells, micronuclei, sticky cells, bridges, c-mitosis (metaphase and anaphase), vacuolated and disturbed cells were frequently observed at various stages of mitosis at all treatments with time- and concentration-independent manner (Table 4, Fig. 4a–i). Goat cells (Fig. 4a) are the most common type recorded after different times at all treatments.

In this study, α-esterase is expressed as three isoforms (Fig. 5); α-EST (esterase) I isoform is present only in treatment of 25% mixed water, and α-EST II is expressed in all treatments and control except treatment of 25%. The isoform α-EST III is expressed in all treatments with different intensity and thickness and treatment of 25% mixed water records the highest intensity and thickness band. The β-esterase was expressed as five isoforms. The isoform β-EST I was absent in treatments of 50% and 100% mixed water with low intensity in treatment of 25% compared to control. The isoform β-EST II disappeared only in treatment of 25% mixed water, and β-EST III was expressed only in treatment of 25% mixed water. The isoforms β-EST IV and β-EST V were expressed with faint bands in all treatments and absent in control.

A great variation in banding pattern profile of ISSR fingerprinting in onion plants was recorded using 10 primers (Table 5). The primers produced a total of 108 bands including 54 polymorphic and 54 monomorphic markers in the control and treated plants with different concentrations of mixed water. Primer UBC 886 recorded the highest percentage of polymorphism (63.64%) with bands in range of size (94–977 bp). Differences in band intensity and thickness were also observed (Figs. 4 and 5). New markers were appeared in the used ISSR primers in the treated plants that were absent in the control such as band formed by primer UBC 808 with size of 280 bp, 3 bands formed by UBC 809 with sizes 210, 344 and 450 bp, and 5 bands formed by UBC 810 with sizes 407, 433, 469, 489 and 994 (Fig. 6). On the other hand, bands appeared in control and were absent in the other treatments such as 2 bands formed by UBC 857 with sizes 286 and 395, a band formed by UBC 884 with size 346, and 4 bands formed by UBC 886 with sizes 230, 337, 836 and 977 bp (Fig. 7).

A cluster analysis (Fig. 8) that was constructed using the arithmetic average (UPGMA) clearly revealed the distinction of plants treated with 100% mixed water and to some extent the plants treated with 50% mixed water from the control plants and plants treated with 25% mixed water.

4 Discussion

Cations and anions measured in mixed water are high compared to control, but were in the permitted limits according to FAO [6] except Mg^{2+} and K^{1+} were above the limits. N, P and heavy metals recorded high values compared to control which probably due to sewage

Table 4 Number of cells examined, mitotic index, percentage of total abnormalities, types and percentage of abnormalities of *Allium cepa* roots after different treatments of mixed water and control

Treatment	No. of cells examined	MI ± SE	% abn		Types and % abnormalities						
					Goat	Micro	Stick	Bridge	Vac. cell	C-mit	Dist
Control	1432	11.69 ± 1.1	1.3	0.0	0.0		0.5	0.0	0.0	0.0	0.8
12 h											
25%	945	12.40 ± 0.9	13.1	26.1	30.8		17.3	9.7	7.8	10.8	7.5
50%	1120	9.70 ± 1.2	14.2	42.4	20.5		20.4	0.0	9.4	7.3	0.0
100%	1620	6.90* ± 1.1	18.2	28.5	0.0		42.6	10.8	13.2	0.0	4.9
24 h											
25%	846	11.10 ± 0.8	6.5	46.5	0.0		16.3	0.0	12.3	16.3	8.6
50%	971	10.10 ± 0.9	13.0	10.3	30.8		20.6	5.1	14.1	8.9	10.2
100%	1063	7.20* ± 1.2	24.1	8.0	24.4		25.0	0.0	9.0	13.3	20.3
36 h											
25%	1680	10.10 ± 1.3	9.6	40.8	9.3		12.3	3.3	10.2	15.8	8.3
50%	1570	9.00 ± 2.0	10.3	35.4	10.5		15.1	5.3	11.5	15.1	7.1
100%	1740	3.50*** ± 1.4	25.2	47.5	11.1		15.2	0.0	7.5	12.4	6.3

* and ***, significant at 0.05 and 0.001 probability levels, respectively

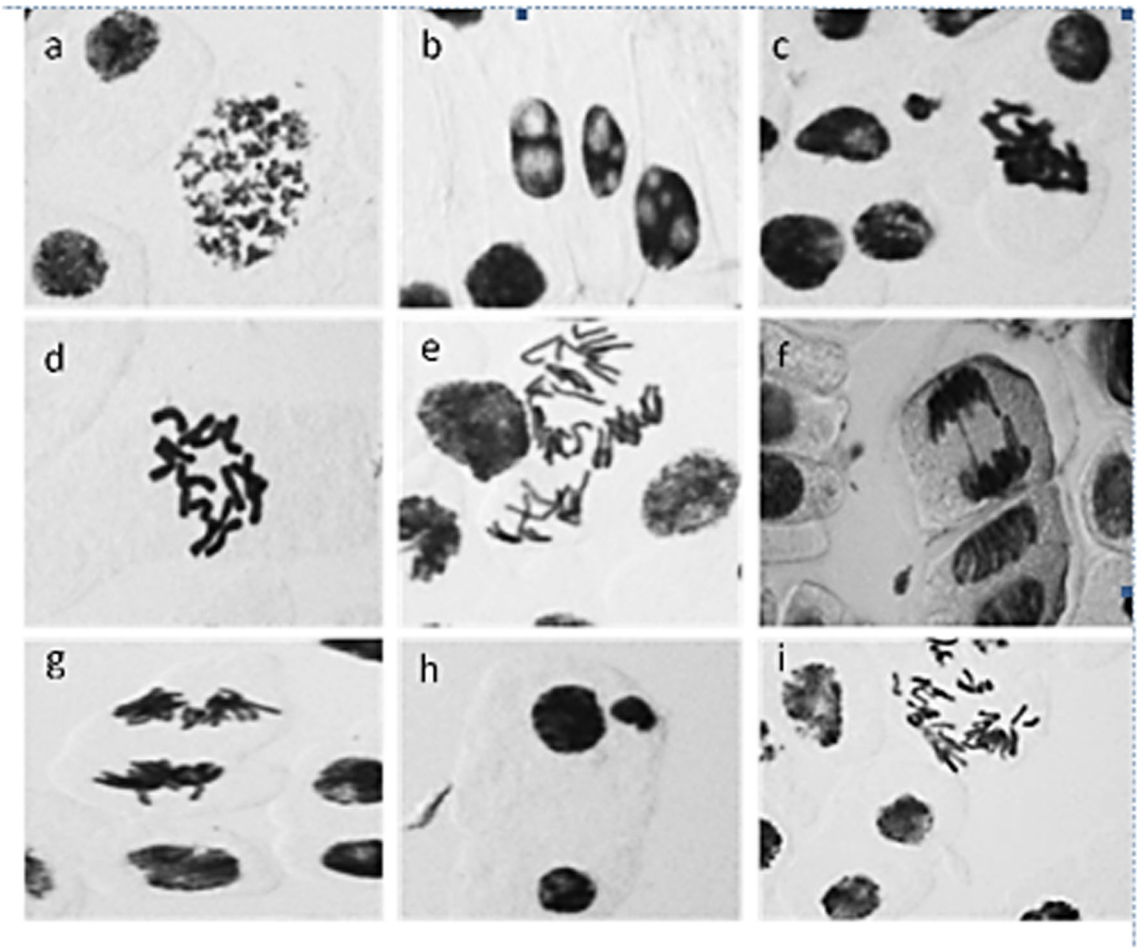

Fig. 4 Different types of mitotic aberrations recorded by treated mixed water on *Allium cepa* root cells. **a** Goat cell, **b** vacuolated cells, **c** sticky cell and micronuclei, **d** c-metaphase, **e** c-anaphase, **f** anaphase, bridge, **g** multipolar cell, **h** micronucleus and **i** disturbed anaphase

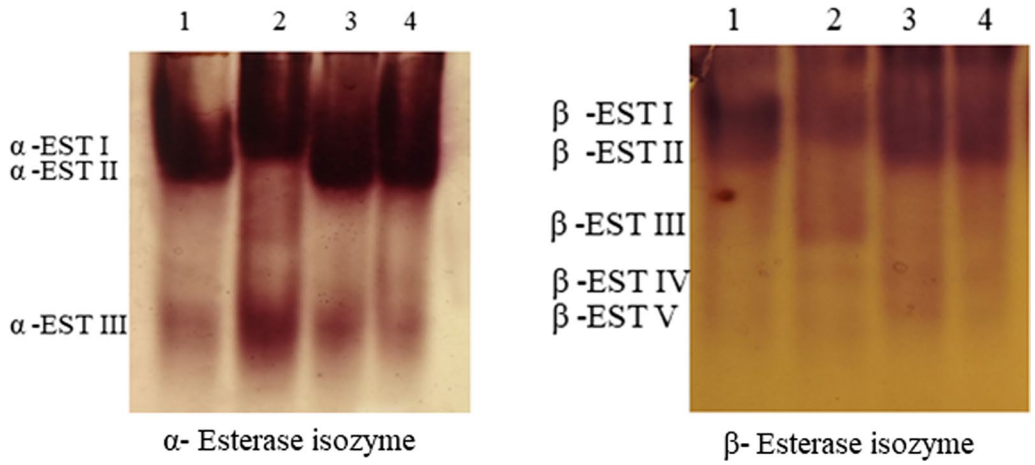

Fig. 5 Expression of α-EST and β-EST zymogrames in roots of *Allium cepa* treated with (1) tap water as control, (2) 25%, (3) 50% and (4) 100% of mixed wastewater at 12th day after germination

Table 5 ISSR profile analysis using 10 primers in *Allium cepa* treated with tap water as control, 25%, 50% and 100% of mixed wastewater at 12th day after germination

No.	ISSR primers name	Primers sequence (5′-3′)	Total bands	Range of size (bp)	Monomorphic bands	Polymorphic bands	% Polymorphism
1	UBC 808	AGAGAGAGAGAGAGAGC	10	210–974	4	6	60
2	UBC 809	AGA GAG AGA GAG AGAGG	7	210–914	3	4	57.14
3	UBC 810	GAGAGAGAGAGAGAGAT	10	218–994	4	6	60
4	UBC 818	CACACACACACACACAG	8	218–1000	5	3	37.5
5	UBC 824	TCTCTCTCTCTCTCTCG	12	218–993	5	7	58.33
6	UBC 826	ACACACACACACACACC	13	121–973	7	6	46.54
7	UBC 836	AGAGAGAGAGAGAGAGYA	15	78–995	8	7	46.67
8	UBC 857	ACACACACACACACACYG	11	107–1008	7	4	36.36
9	UBC 884	HBH AGA GAG AGA GAG AG	11	99–1008	7	4	36.36
10	UBC 886	VDV (CT)7	11	94–977	4	7	63.64
Total			108		54	54	50.25%

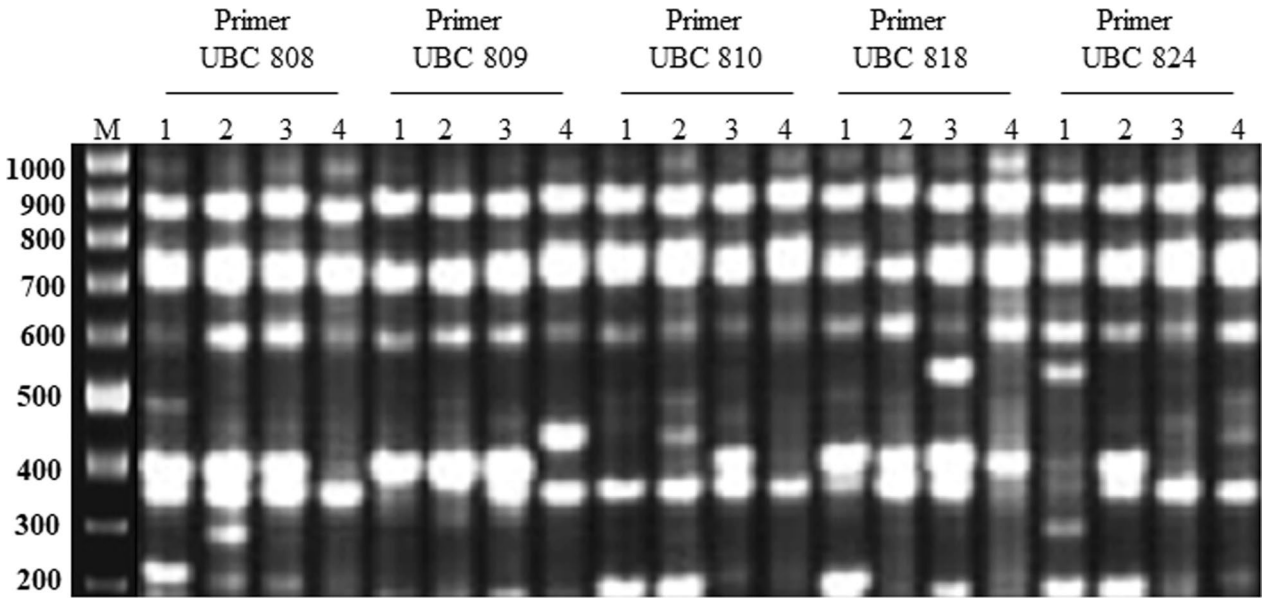

Fig. 6 ISSR profile of *Allium cepa* treated with (1) tap water as control, (2) 25%, (3) 50% and (4) 100% of mixed wastewater at 12th day after germination, using 5 different primers. M = Standard DNA molecular size marker

effluents and this to some extent affects water content and causes a severe problem to crop plants when used for irrigation.

Root length significantly decreased in mixed water as the concentration increased after twelve days of germination that was consistent with Kassa et al. [25] and Admas and Kerisew [1]. This reduction might be caused by accumulation of metals in roots than other plant parts, as previously indicated by [31]. Also, shoot length measurement significantly decreased as the concentration increased and these results were consistent with Goli and Sahu [16] and Kapil and Mathur [24]. Hajihashemi et al. [18] reported that reduction in growth may be due to reduction of the photosynthetic characteristics, nutrient concentrations and carbohydrates in treated plants. With increasing effluent concentrations of industry wastewater, a gradual decline in the germination of seed and seedling growth of *Vigna radiata* was observed by Kothari et al. [26]. Shoot dry weight recorded nonsignificant reduction in all treatments compared to control as previously stated by Goli and Sahu [16] and Kamlesh [23]. The root length can serve as a crucial tolerance index due to suppression of root elongation is the first noticeable effect of metal toxicity [19]. Different treatments of

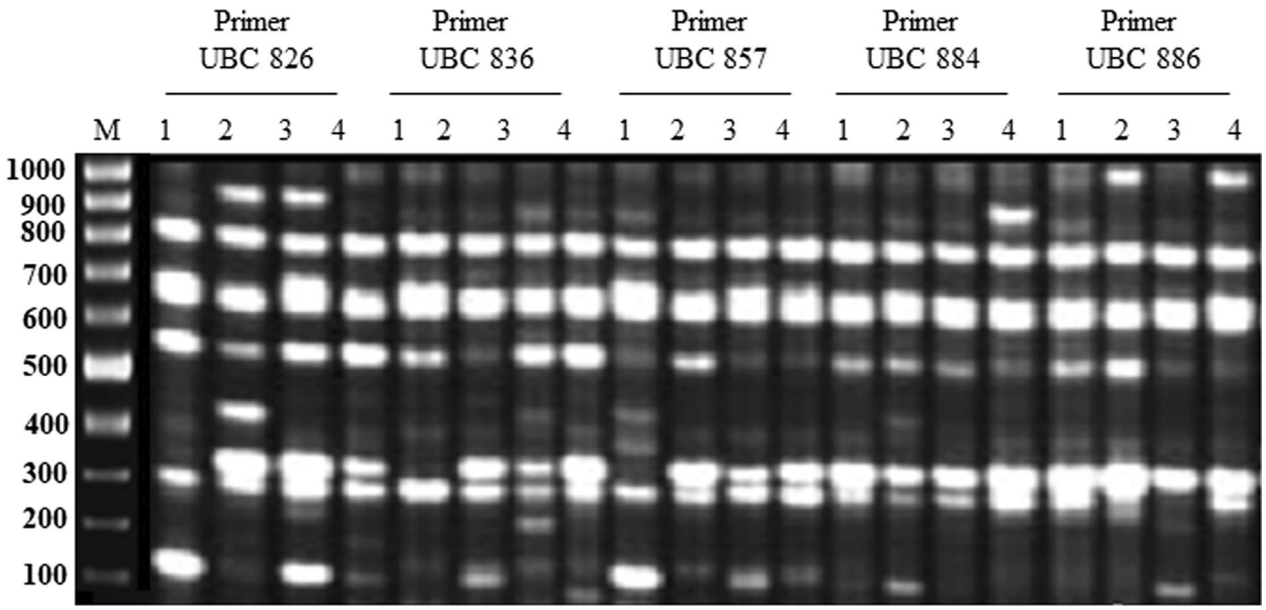

Fig. 7 ISSR profile of *Allium cepa* treated with (1) tap water as control, (2) 25%, (3) 50% and (4) 100% of mixed wastewater at 12th day after germination, using 5 different primers M = Standard DNA molecular size marker

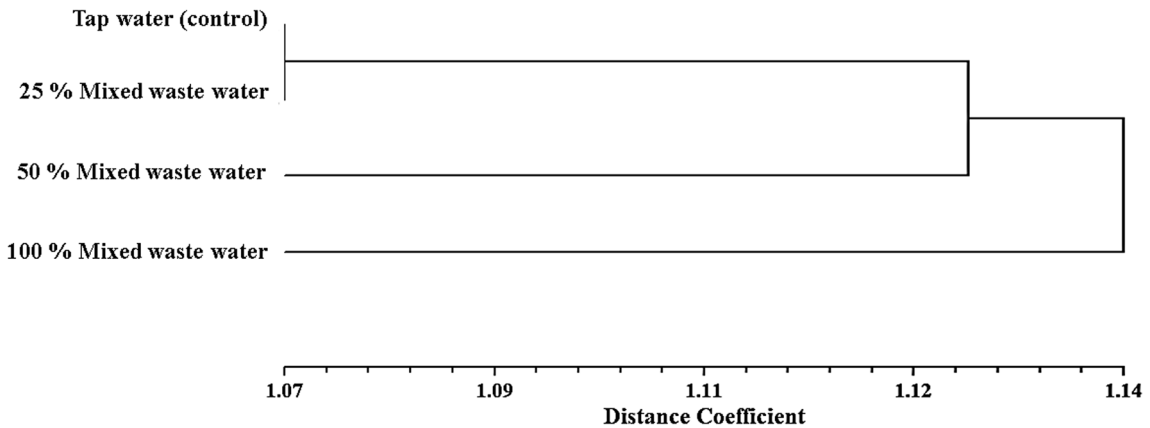

Fig. 8 Dendrogram illustrating differentiation of *Allium cepa* treated with tap water as control, 25%, 50% and 100% of mixed wastewater at 12th day after germination based on polymorphism in ISSR fingerprinting

mixed water showed highly significant low values of tolerance index that agreed with Mehta and Bhardwaj [36] and Kamlesh [23].

For all living organisms, the mitotic index is regarded as an acceptable indicator of cytotoxicity [44]. Abnormalities that were recorded in control (1.3%) may be due to hard handling of slides during squash technique rather any other effects [20, 37]. MI of 25% concentration was, approximately, the same as in control that it was suggested this concentration might be suitable for growth and act as fertilizer and agreed with Maxim and Andrey [35], Dourado et al. [14] and Cresenico et al. [12]. The lowest value recorded for MI by concentration of 100% at 36-h treatment is time and dose dependent, and percentage of mitotic aberrations increased as the concentration increased and the time prolonged as previously recorded by Labeeb [27], Mohamad [37], Cresenico et al. [12] and Admas and Kerisew [1]. This result proves the harmful and toxic effect of mixed water in this pool, especially when used in its present form for long time.

Different types of abnormalities such as goat cells, micronuclei, sticky cells, bridges, c-mitosis (metaphase and anaphase), vacuolated and disturbed cells were frequently observed at various stages of mitosis at all

treatments with time- and concentration-independent manner. Goat cells are the most common type recorded after different times at all treatments; to our knowledge, this aberration was not previously recorded by any treatment with polluted water. To some extent, this may be due to the double action of mixed water rich in different types of pollutants and heavy metals from either drainage water or sewage effluents. The high percentage of goat cells may also be due to interaction of some abiotic factors [3, 4]. The other types of aberrations were previously recorded by many authors using different plants irrigated with polluted water [1, 21, 25, 27, 37].

The results of α- and β-esterase isozymes illustrate that concentration of 25% mixed water induces different profiles for expression of both α- and β-esterase from other treatments that may be agree with our previous suggestion that this concentration is suitable for growth and may act as fertilizer. The variation in expression of esterase isozymes in our study may be a sign that plant defense systems have been activated to withstand the stress caused by heavy metals and other contaminants present in these effluents as previously stated by Mukherjeea et al. [38], Mattar et al. [34] and Labeeb et al. [29].

A great variation in banding pattern profile of ISSR fingerprinting in onion plants was recorded, and differences in band intensity and thickness were also observed. Following exposure to environmental pollutants, polymorphism of molecular markers created by DNA fingerprinting using the randomly amplified polymorphic DNA (RAPD) and ISSR linked genotoxicity to genetic variation [47]. Also, Bajpai et al. [8], Bakry et al. [9] and Badr et al. [7] used ISSR-PCR technique to investigate genotoxicity. Changes in DNA banding profiles indicate DNA modifications from single base alterations to chromosomal rearrangements [5], possibly because of the mutagenicity and toxicity of the heavy metals in the effluent [2]. Also, the stress causes a quick and temporary overproduction of reactive oxygen species (ROS), which damages DNA in plants [7]. New markers were appeared in the used ISSR primers in the treated plants that were absent in the control and bands appeared in control and were absent in the other treatments. The alternation of DNA profiles including loss or appearance of some bands occurs as a result of changes in oligonucleotide priming sites that impact the activity and interaction of DNA polymerase with changed DNA [11, 40, 42].

A cluster analysis clearly revealed the distinction of plants treated with 100% mixed water and to some extent the plants treated with 50% mixed water from the control plants and plants treated with 25% mixed water. These results were consistent with Kamal et al. [22]. Concentration of 25% mixed water is the most relative to control (tap water) that may be agree with our previous suggestion that this concentration is suitable for growth and may act as fertilizer.

As this pool is frequently considered an easy source of water for irrigation, as well as the ignorance of many farmers, this study illustrates how much toxicity of this water on crops and vegetables when used directly or indirectly or incorporated in food chain of the ecosystem as previous studies of plants were affected by industrial waste [53, 55].

5 Conclusion

Different treatments of mixed water showed significant decrease in root and shoot lengths as the concentration increased and highly significant low values of tolerance index. Different types of abnormalities were frequently observed at various stages of mitosis at all treatments with time- and concentration-independent manner and goat cells were the most common type. MI of 25% concentration of mixed water was, approximately, the same as in control, this concentration induced different profiles for expression of both α- and β-esterase, and a cluster analysis based on polymorphism in ISSR fingerprinting revealed the distinction of plants treated with it and the control plants from those treated with high concentrations. It was suggested that concentration of 25% mixed water may be suitable for growth and act as fertilizer. Water from this pool may be genotoxic for *Allium cepa* plants at early growth if it is used for irrigation in its present form and usage of this wastewater for agricultural purposes may be harmful and must be partially treated and biologically tested before use.

Abbreviations

ISSR	Inter-simple sequence repeat
EC	Electric conductivity
MI	Mitotic index
ROS	Reactive oxygen species
PCR	Polymerase chain reaction
ANOVA	Analysis of variance
UPGMA	Unweighted pair group method with arithmetic mean
Micro	Micronuclei
Stick.	Stickiness
Vac. cell	Vacuolated cell
C-Mito	C-Metaphase
Dist.	Disturbed chromosome
EST	Esterase
RAPD	Randomly amplified polymorphic DNA
FAO	Food and Agricultural Organization

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ASE was involved in experimental investigations, data interpretation, and reviewing and editing the manuscript. SAH was responsible for conceptualization, and reviewing and editing the manuscript. ML contributed to experimental investigations, writing of the original draft, data interpretation, and reviewing and editing the manuscript. All authors read and approved the final version.

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