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Research Article

Genotoxic effect of heavy metals on *Ceratophyllum demersum* L. using RAPD markers

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Abstract
The genotoxicity of Cd, Ni, and Pb was studied on *Ceratophyllum demersum* L. in three concentrations of 2.5, 5, and 7.5 mg/L. The results showed genotoxic as descending order of Cd > Pb≥ Ni. All concentrations of Cd, Ni, and Pb with the interference of three-elements showed genotoxic effects. Three out of five primers of the RAPD markers showed replication show replication. The total number of bands was 288, polymorphic bands of 11 and monomorphic band of 9 and rare bands of 4 with the polymorphism percentage of 46.36%. The results of the UPGMA for the twenty-two treatments using the RAPD marker showed two main clusters. It was found that the higher concentrations of the heavy metals have the lower the similarity ratio with the control treatment.

Keywords: Aquatic plants, Genotoxicity, RAPD, Genetic divergence, Heavy metals

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Introduction

One of the main interests of this century is the conservation of environmentAL quality. Aquatic ecosystems are indirectly or directly ending as destinations of many pollutants (Cardwell et al. 2002). Phytoremediation is used to remove heavy metal pollutants or decreases organic matter from water (Mishra & Tripathi 2008). The accumulation of heavy metals in plants and animals causes biochemical, physiological, and genetic changes (Dhir et al. 2004; Pavlikova et al. 2008; Jawad et al. 2021). *Ceratophyllum demersum* L. grows in muddy, shallow water bodies at depressed light intensities. It is a rootless, submerged, free-floating plant with a high ability for vegetative propagation even under depressed nutritional conditions worldwide. It is characterised by removing excess heavy metals from water. It is useful for use in an equilibrated biological aquatic system (Foroughi et al. 2010). *Ceratophyllum demersum* is a biological indicator of water pollution with cadmium and lead (Jawad et al. 2018). The effects are more in the higher concentrations and prolonged duration of exposure (Bhowmik 2000; Patra 2004).

Random Amplified Polymorphic DNA (RAPD) methods are mostly used to indicate the genetic relationships. Banding patterns can be scored to detect DNA damage and mutations (Atienzar et al., 1999). RAPD is also a tool to assess the effects of toxicants on organisms and to diagnose genotoxicity. The band's absence, presence, and intensity are linked to DNA damage. The cadmium is a genotoxic heavy metal since it directly effects DNA function and structure (Liu et al. 2009; Cambier et al. 2010). The present study was conducted to estimate the genotoxicity of cadmium, nickel, and lead on *C. demersum* using RAPD.

Materials and Methods

Collection of plant samples: *Ceratophyllum*

demersum samples were collected randomly in December 2020 from the Hussainiya River in Karbala, Iraq. The plant was washed in river water several times to get rid of the suspended substances. The collected samples were placed in plastic tanks, and river water was added to tanks to preserve the plant. Then, the plants were transferred to the laboratory, and washed with tap water several times. To the plastic tanks with a capacity of 30 liters, 20 liters of distilled water and 500g of the plant sample were added. Three heavy metals, include lead (pb), cadmium (cd) and nickel (Ni) in the form of salts in concentrations of 2.5, 5 and 7.5mg/L, respectively, were used. Treatments were as: pb, Ni, cd, pb+Ni, cd+Ni, Pb+cd and Pb+cd+Ni each with three replicates.

Genomic DNA isolation for PCR analysis: DNA was isolated from *C. demersum* of each treatment and control group using the modified CTAB method (Khan et al., 2007). DNA purity and concentration were determined by bio drop equipment. DNA quality was determined by agarose gel electrophoresis using ethidium bromide staining. Using 5 RAPD primers (Table 1), PCR amplification was done. The amplification on the Primus PCR was don as denaturation at 95°C for 5min, denaturation at 95°C for 30s, annealing at 37°C for 30s, extension 1min at 72°C and final extension 5min at 72°C.

Statistical analysis: The resulting RAPD bands were successful for 3 primers of RAPD and were notarized as absent = 0 and present = 1. These bands were used to construct the dendrogram by PASTA3 program to calculate polymorphism%, Discriminatory value%, Primer efficiency for each primer based on Hunter & Gaston (1988) and Graham & McNicol (1995).

Results and Discussion

RAPD analysis: DNA was isolated from the control sample and all treatments and, the purity ranged 1.8-1.9 at wavelength 260 and 280nm. The multiplication results are represented by the bands that differ in their numbers and molecular sizes according to the primer used, resulting from the difference in the number of complementary sites for that primer. Three primers of the RAPD index gave different PCR products in terms of numbers and sizes, ranged 100 to 900 base pairs.

Analysis of the primer multiplication OP-A05: The nine bands were formed ranging from 100-700pb including four polymorphic bands, three monomorphic bands and two rare bands for primer OP-A05 (Table 2). The total bands were 102 in all treatments and control groups. The highest number of multiplied was six bands in the treatment of Pb and Cd and the interaction of Cd and Ni, and the interaction of three elements with a concentration of 7.5mg.L^{-1}. We found the similarity of the results between the control and the single treatment samples for Pb and Ni (Fig. 1). The results showed four bands, while a band of 250pb appeared with a lacking band of 500pb in treatments of Cd for concentrations of 2.5 and 5mg.L^{-1}. For the treatment of Cd at a concentration of 7.5mg.L^{-1}, two bands of 250 and 300pb were formed. The plant may show some symptoms when the concentration of Cd increases in its tissue, such as yellowing and wilting of plant leaves due to Cd stress, as found by Ismael et al. (2019).

Figure 2 shows the results of the OP-A05 primer for the two elements, the similarity of the number of bands for the interaction Pb+Ni observed in all concentrations with the appearance of a new band of 350pb. The treatment of Pb+Cd for a concentration of 2.5mg.L^{-1} showed two new bands of 350 and 600pb with the absence of the band of 500pb, while the interaction of Pb and Cd for 5mg.L^{-1} showed two bands of 250 and 600pb and a new band of 250pb and a rare band of 150pb. For the interaction between Cd and Ni in 5mg.L^{-1}, a new band of 250pb appeared, while the interaction treatment for concentration 2.5 and 7.5mg.L^{-1} two new bands of 250 and 300pb were formed; whereas band of 500pb at 2.5mg.L^{-1} not formed. This indicates the genotoxic effects of Cd on the plant's DNA, as high concentrations of Cd have a toxic effect on the levels of gene expression as well as negatively affect the phenotypic and physiological

Table 1. List of the RAPD Primers used in the study.

	Primer	Primer sequence	Reference
1	Op-05	5'-AGGGGTCTTG-3	
2	OP-15	5′-*TTC CGA ACC* C-3′	(Soliman et al.
3	Op-01	5'-CCGCATCTAC-3'	2009; Gupta &
4	Op-03	5'-GTCGCCGTCA-3'	Sarin 2009).
5	Op-15		

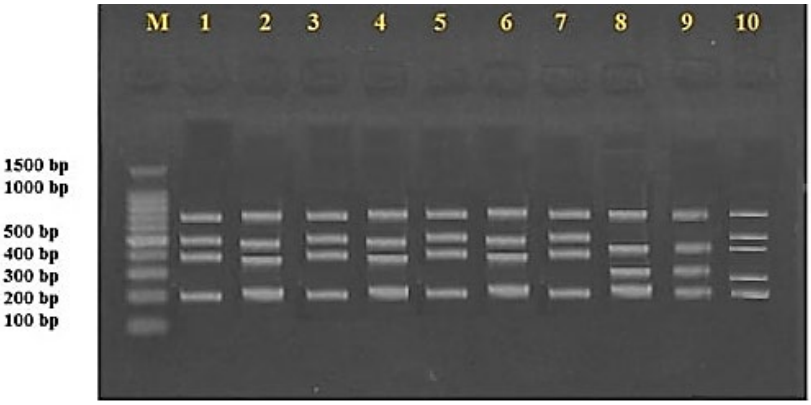

Fig.1. shows the results of primer OP-A5 on agarose gel at a concentration of 1.5% for 45 min with DNA ladder M (100-1500bp) for: 1-Control, 2-Pb (2.5mg/L), 3-Pb (5mg/L), 4- Pb (7.5mg/L), 5- Ni (2.5mg/L), 6- Ni (5mg/L), 7- Ni (7.5mg/L), 8- Cd (2.5mg/L), 9- Cd (5mg/L), 10- Cd (7.5 mg/L).

Table 2. The Total number of the amplified fragments and the number of polymorphic bands to RAPD-PCR marker.

Name of primer	Sequence (5'–3′)	Total number of bands	Polymorphic bands	Monomorphic bands	Percentage polymorphism	rare bands	primer efficiency	discriminatory value	G +C content
OP-A5	5'-AGGGGTCTTG-3′	102	4	3	44.44	2	0.040	36.36	60
Op-c01	CCGCATCTAC-3' 5'-	78	4	2	57.14	1	0.051	36.36	60
Op-D03	GTCGCCGTCA-3' 5'-	108	3	4	37.50	1	0.027	27.27	70
Op-A15	5′-*TTC CGA ACC* C-3′	-----		-----	-----	-----	-----	-----	60
Op-E15		-----		-----	-----	-----	-----	-----	

characteristics of the plant and this is consistent with studies of Gzyl et al. (2015) and Jinadasa et al. (2016).

Figure 3 shows the results of primer OP-A05 for the interaction of three elements. The triple overlap coefficients of the elements for all concentrations showed a band of size 300pb and the lacking band of 500pb in the treatment 2.5mg/L, and the a rare band of 100pb at the concentration 7.5mg.L^{-1}. Exposure to toxic heavy metals such as lead and cadmium increases the production of free radicals. Reaction Oxygen Species (ROS) cause DNA damage in a variety of plants, and this was consistent with pervious findings (Silveira et al. 2017; Cao et al. 2018). The polymorphism ratio for this primer was 44.44%, and it efficiency 0.040, while its discriminatory value was 36.36%.

The results of primer multiplication Op-C01: The results of primer Op-C01 showed seven main bands ranged 200-600pb, including four polymorphic bands, two monomorphic bands, and one rare band. Total bands were 78 in all treatments with control group. The highest number bands were five in the treatment of Cd with concentration of 5mg.L^{-1}. Figures 4-6 show the PCR amplification of the primer Op-C01 in all treatments and control group. Treatment of Pb at concentration of 2.5, 5 and 7.5mg.L^{-1}, as well as Ni 2.5, 5 and 7.5mg.L^{-1} and the

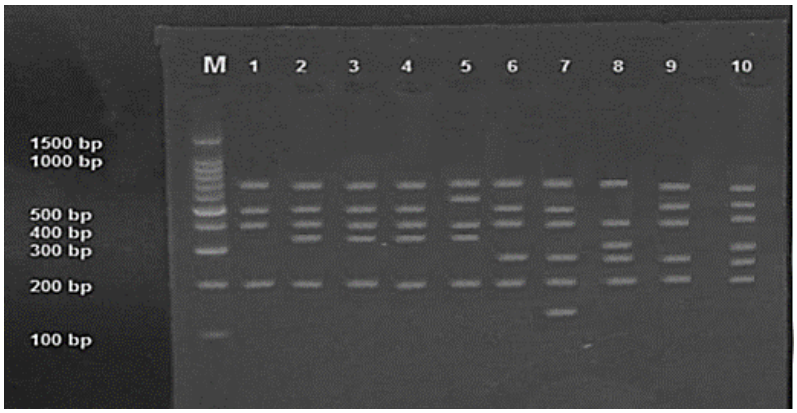

Fig.2. The results of primer OP-A5 on agarose gel at a concentration of 1.5% for 45min with DNA ladder M (100-1500bp) for: 1- Contro, 2- Pb+Ni (2.5mg/L)), 3- Pb+Ni (5mg/L), 4- Pb+Ni (7.5mg/L), 5- Pb+Cd (2.5mg/L), 6- Pb+Cd (5mg/L), 7- Pb+Cd (7.5mg/L), 8- Cd+Ni (2.5mg/L), 9- Cd+Ni (5mg/L), and 10- Cd (7.5mg/L).

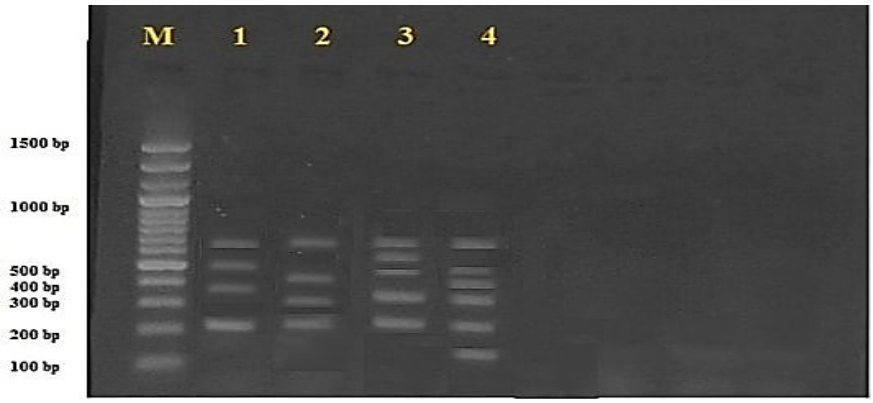

Fig.3. the results of primer OP-A5 on agarose gel at a concentration of 1.5% for 45min with DNA ladder M (100-1500bp) for 1- Control, 2- Pb+Cd+Ni (2.5mg/L), 3- Pb+Cd+Ni (5mg/L), and 4- Pb+Cd+Ni (7.5mg/L).

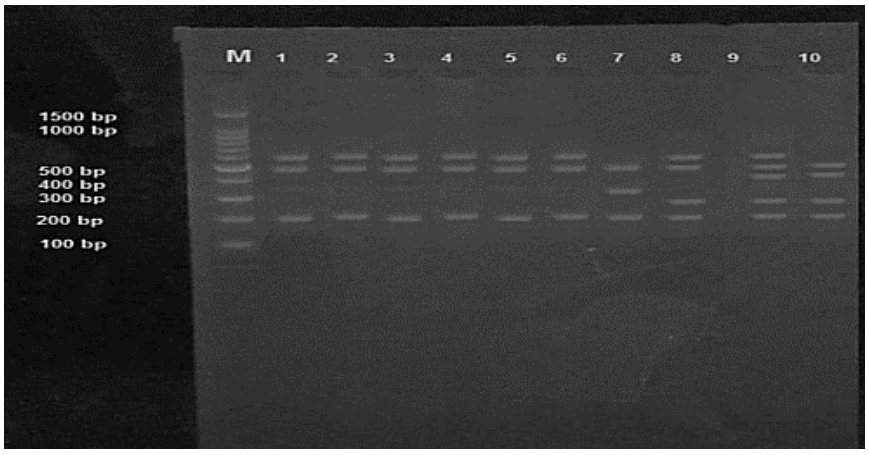

Fig.4. The results of primer OP-C01 on agarose gel at a concentration of 1.5% for 45min with DNA ladder M (100-1500bp) for: 1- Control, 2- Pb (2.5mg/L), 3-Pb (5mg/L), 4- Pb (7.5mg/L), 5- Ni (2.5mg/L), 6- Ni (5mg/L), 7- Ni (7.5mg/L), 8- Cd (2.5mg/L), 9- Cd (5mg/L), and 10- Cd (7.5mg/L).

Pb and Ni of 2.5 and 5mg.L^{-1} are similar to the control treatment with three bands. In contrast, while the Ni of 7.5mg.L^{-1}, the interaction of Cd and Ni of 5mg.L^{-1}, and the interaction of three elements in the concentration of 2.5mg.L^{-1}, the results showed a new band of 300pb and the lacking band of 600pb in addition to the other bands. A new band of 250pb appeared in the Cd with a concentration of 2.5mg.L^{-}

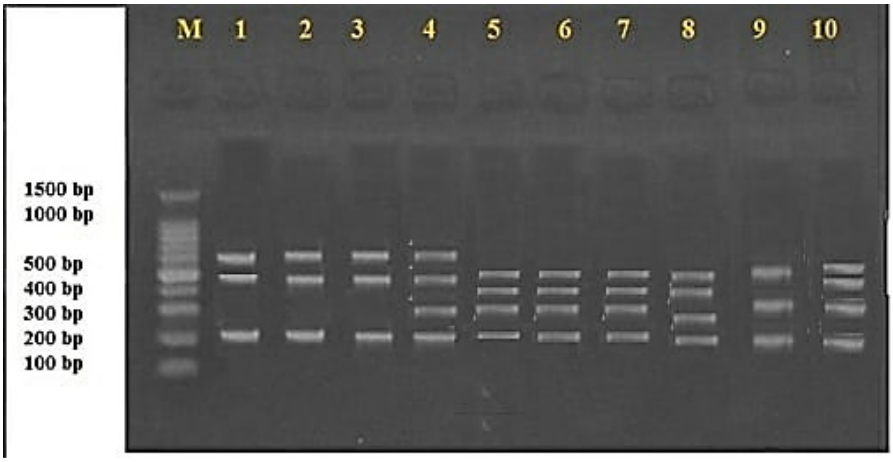

Fig.5. The results of primer OP-C01 on agarose gel at a concentration of 1.5% for 45min with DNA ladder M (100-1500bp) for: Control, 2- Pb+Ni (2.5mg/L), 3- Pb+Ni (5mg/L), 4- Pb+Ni (7.5mg/L), 5- Pb+Cd (2.5), 6- Pb+Cd (5),7- Pb+Cd (7.5), 8- Cd+Ni (2.5mg/L), 9- Cd+Ni(5mg/L) and 10- Cd (7.5mg/L).

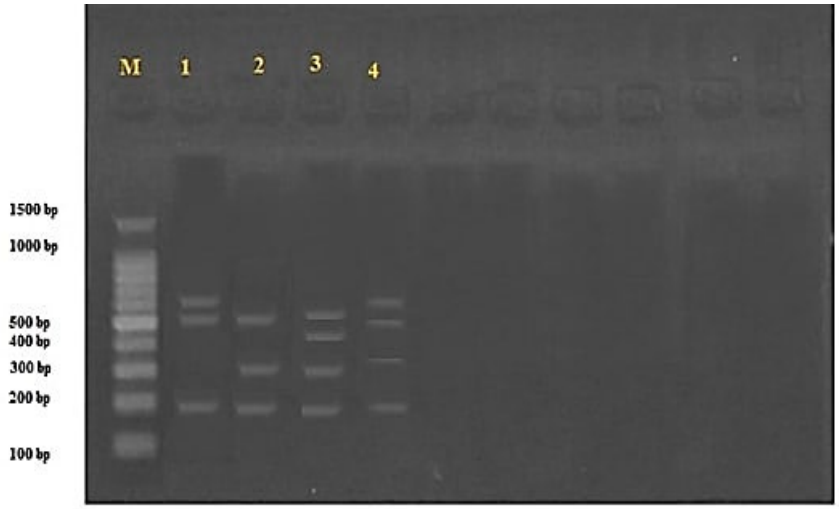

Fig.6. The results of primer OP-C01 on agarose gel at a concentration of 1.5% for 45min with DNA ladder M (100-1500bp) for: 1- Control, 2- Pb+Cd+Ni (2.5mg/L), 3- Pb+Cd+Ni (5mg/L) and 4- Pb+Cd+Ni (7.5mg/L).

[1] and the Cd treatments for concentrations of 5 and 7.5mg.L^{-1}, the two new bands of 250 and 400pb with the lacking of 600pb band were formed in Cd treatment 7.5mg.L^{-1}. the Cd causes DNA damage to many plants (Huybrechts et al. 2019).

A new 300pb band appeared in the interference treatment of Pb and Ni (7.5mg.L^{-1}) and the interference treatment of Pb and Cd for all concentrations with two new bands of 300 and 400pb, and lacking band of 600pb. The interference treatment of Cd and Ni for concentrations of 2.5 and 7.5mg.L^{-1} and the interaction of three elements for concentration 5 and 7.5mg.L^{-1}. The results also showed a new band of 350pb. High concentrations of Cd have a strong inhibitory effect on the phenotypic characteristics (Liu et al. 2009). The polymorphism ratio of this primer was calculated as 57.14%, and efficiency as 0.051, and discriminatory of 36.36%.

The results of primer multiplication OP-D03: The results of the primer OP-D03 were shown producing eight main bands ranging 100-900pb, which included three polymorphic bands, four monomorphic bands, and one rare bands. The total number of bands was 108 all treatments with control. The highest number of bands was seven bands in the interaction treatment of Pb and Cd with a concentration of 5mg.L^{-1} and the interaction of three elements with a concentration of 7.5mg.L^{-1}. Figures 7-9 show the PCR amplification

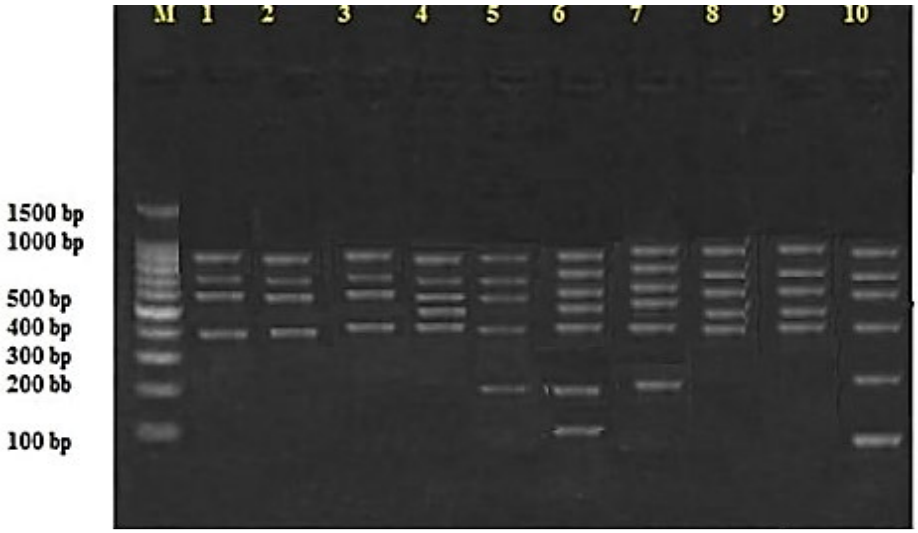

Fig.7. The results of primer OP-D03 on agarose gel at a concentration of 1.5% for 45 min with DNA ladder M (100-1500bp) for: 1- Control, 2-Pb (2.5mg/L), 3-Pb (5mg/L), 4- Pb (7.5mg/L), 5- Ni (2.5mg/L), 6- Ni (5mg/L), 7- Ni (7.5mg/L), 8- Cd (2.5mg/L), 9- Cd (5mg/L), 10- C.d(7.5mg/L).

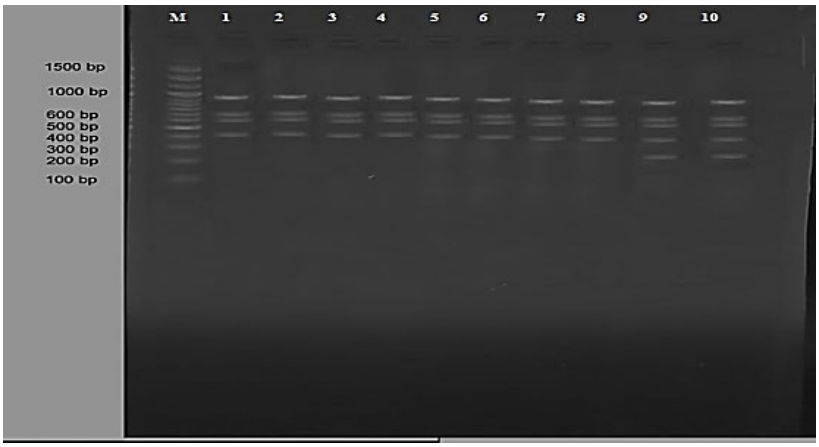

Fig.8. The results of primer OP-D03 on agarose gel at a concentration of 1.5% for 45min with DNA ladder M (100-1500bp) for: 1 Contro, 2- Pb+Ni (2.5mg/L), 3- Pb+Ni (5mg/L), 4- Pb+Ni(7.5mg/L), 5- Pb+Cd(2.5mg/L), 6- Pb+Cd (5mg/L),7- Pb+Cd (7.5mg/L), 8- Cd+Ni (2.5mg/L), 9- Cd+Ni (5mg/L), 10- Cd (7.5mg/L).

of the OP-D03 primer for all treatments with control, as the results showed that all concentrations of Pb and Ni single treatments in addition to the Cd treatment with a concentration of 2.5mg.L^{-1}. The interaction treatment of Pb and Ni for concentrations of 2.5 and 5mg.L^{-1} was similar to the control one.

For the Cd treatment for concentrations of 5 and 7.5mg.L^{-1} and the interaction treatment of Pb and Cd for the concentration of 2.5mg.L^{-1}, the results showed the new band of 200pb. A new band of 500pb was appeared in the interaction treatments of Pb and Ni for concentration of 7.5mg.L^{-1} and Cd and Ni for concentrations of 2.5 and 5mg.L^{-1}. For the interaction treatments of Pb and Cd for concentration of 5mg/L^{-1} and the interaction of three elements for concentration of 7.5mg.L^{-1}, the results showed the three new bands of 100, 200 and 500pb. While two new bands of 200 and 500pb were observed in the interaction treatments of Pb and Cd for the concentration of 7.5mg.L^{-1} and the interaction treatments of three elements for concentrations of 2.5, and 5mg.L^{-1}. For the interaction treatments of Cd and Ni 7.5mg.L^{-1}, the results showed the new band size 100pb and a rare band of 300pb in addition to the main bands. Heavy metals exceeding the permissible limit in the environment lead to the emergence of

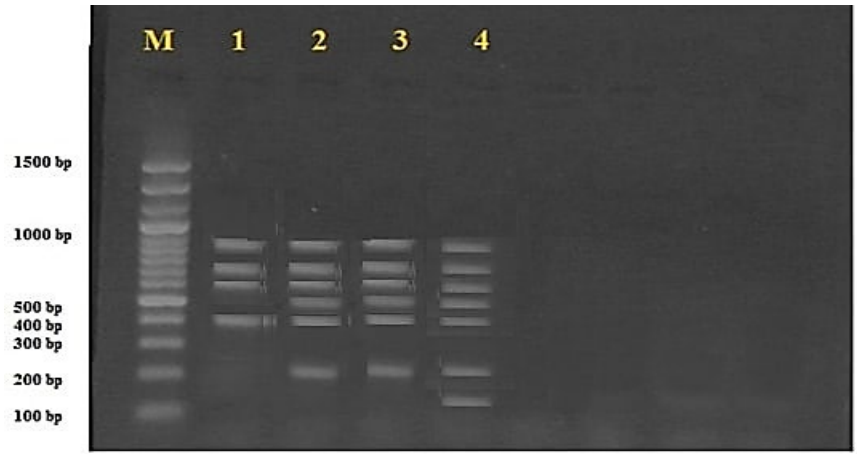

Fig.9. The results of primer OP-D03 on agarose gel at a concentration of 1.5% for 45min with DNA ladder M (100-1500bp):1-Control, 2- Pb+Cd+Ni (2.5mg/L), 3- Pb+Cd+Ni (5mg/L) and 4- Pb+Cd+Ni (7.5mg/L).

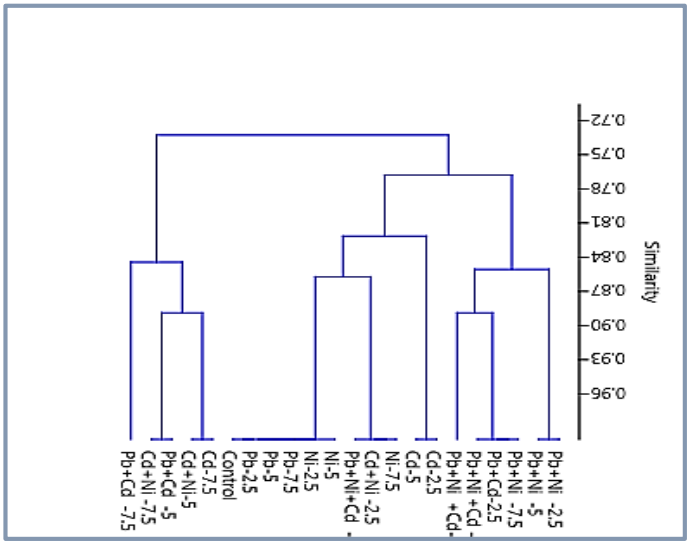

Fig.10. The UPGMA Dendrogram of *Ceratophyllum demersum* L. treatment with 3 different concentrations (2.5, 5 and 7.5mg/L) of pb, cd, Ni, pb+Ni, cd+Ni, Pb+cd, Pb+cd +Ni and control based on RAPD markers.

various toxicity symptoms to plants as a result of the influence of DNA, which is reflected in various physiological changes (Gautam et al. 2017). The polymorphism ratio for this primer was calculated as 37.5%, the efficiency as 0.027, and discriminatory as 27.27%.

The results of the UPGMA analysis for the twenty-two treatments showed two main groups (Main Cluster). The first main group include two sub-cluster groups with 100% similarity between the control treatment and the Pb treatments for all concentrations and the two Ni treatments for concentrations of 2.5 ad 5mg.L^{-1}, while the percentage of similarity decreased to 85% in each of the Ni treatment with a concentration of 7.5mg.L^{-1}, Cd treatment for concentrations of 2.5 and 5mg.L^{-1}, the interaction treatment of Pb and Ni for concentrations of 2.5 and 5mg.L^{-1}, the interaction treatment of Cd and Ni for a concentration of 2.5mg.L^{-1} and the triple overlap treatment for with a concentration of 2.5mg.L^{-1}. The higher concentrations used for the treatments, the lower the similarity ratio was observed, as it reached to75% in the Cd treatment for concentration of 7.5mg.L^{-1}, the interaction treatment of Pb and Ni for concentration of 7.5mg.L^{-1}, the interaction treatment of Pb and Cd for the concentration 2.5mg.L^{-1} and the triple interaction treatment to concentration of 5mg.L^{-1}. The difference in the DNA sequence due to genotoxic factors of heavy metals to which the plant is exposed in quantities exceeding the permissible somewhat led to its reflection in the number of bands, such as the multiplication of new bands or the disappearance of Other band and this result is in agreement with Aslam et al. (2014) and Dogan et al. (2016).

References

Ismael, M.A.; Elyamine, A.M.; Moussa, M.G.; Cai, M.; Zhao, X. & Hu, C. 2019. Cadmium in plants: uptake, toxicity, and its interactions with selenium

fertilizers. Metallomics 11(2): 255-277.

Aslam, R.; Ansari, M.Y.K.; Choudhary, S.; Bhat, T.M. & Jahan, N. 2014. Genotoxic effects of heavy metal cadmium on growth, biochemical, cyto-physiological parameters and detection of DNA polymorphism by RAPD in *Capsicum annuum* L.-An important spice crop of India. Saudi Journal of Biological Sciences 21(5): 465-472.

Atienzar, F.A.; Conradi, M.; Evenden, A.J.; Jha, A.N. & Depledge, M.H. 1999. Qualitative assessment of genotoxicity using random amplified polymorphic DNA: comparison of genomic template stability with key fitness parameters in Daphnia magna exposed to benzo[a] pyrene. Environmental Toxicology and Chemistry 18(10): 2275-82.

Bhowmik, N. 2000. Cytotoxic effects of lead compounds on plant systems. Ph.D. dissertation. University of Calcutta, Calcutta.

Cambier, S.; Gonzalez, P.; Durrieu, G. & Bourdineaud, J.P. 2010. Cadmium-induced genotoxicity in zebra fish at environmentally relevant doses. Ecotoxicology and Environmental Safety 73; 312-319.

Cao, X.; Wang, H.; Zhuang, D.; Zhu, H.; Du, Y.; Cheng, Z. & Liu, W. 2018. Roles of MSH2 and MSH6 in cadmium-induced G2/M checkpoint arrest in Arabidopsis roots. Chemosphere 201: 586-594.

Cardwell, A.J.; Hawker, D.W. & Greenway, M. 2002. Metal accumulation in aquatic macrophytes from Southeast Queensland, Australia. Chemosphere 48(7): 653-63.

Dogan, I.; Ozyigit, I.I,; Tombuloglu, G.; Sakcali, M.S.; Tombuloglu, H. 2016. Assessment of Cd-induced genotoxic damage in Urtica pilulifera L. using RAPD-PCR analysis. Biotechnology & Biotechnological Equipment 30(2): 284-291

Foroughi, M.; Najafi, P.; Toghiani, A. & Honarjoo, N. 2010. Analysis of pollution removal from wastewater by *Ceratophyllum demersum*. African Journal of Biotechnology 9(14): 2125-2128.

Gautam, S.; Rathoure, A.K.; Chhabra, A. & Pandey, S.N. 2000. Effects of nickel and zinc on biochemical parameters in plants-a review. Octa Journal of Environmental Research 5(1):14-21.

Graham, J. & McNicol, R.J. 1995. An examination of the ability of RAPD markers to determine the relationships within and between *Rubus* spp. Theoretical and Applied Genetics 90: 1128-1132.

Gzyl, J.; Chmielowska-Bąk, J.; Przymusiński, R. & Gwóźdź, E.A. 2015. Cadmium affects microtubule organization and post-translational modifications of tubulin in seedlings of soybean (*Glycine max* L.). Frontiers in Plant Science 6: 937.

Hassan, J.; Jawad, Zeina Thamer, A.; Alrufaye, H.J.A. 2021. The concentration of some trace metals elements in water and tilapia zilli, Coptodon zillii, gills and muscle in rearing ponds of Kerbala region, Iraq. Iranian Journal of Ichthyology (Special issue 1): 255-266

Hunter, P.R. & Gaston, M.A. 1988. Numerical index of discriminatory ability of simpson's index of diversity. Journal of Clinical Microbiology 26:2465-2466.

Huybrechts, M.; Cuypers, A.; Deckers, J.; Iven, V.; Vandionant, S.; Jozefczak, M. & Hendrix, S. 2019. Cadmium and plant development: an agony from seed to seed. International Journal of Molecular Sciences 20(16): 3971.

Jawad MM, Abed EH, Oudah HK. 2018. Using of *Ceratophyllum demersum* L. for lead and cadmium pollution removal by columns technology. Journal of Bacteriology and Mycology 6(1): 19-21.

Jinadasa, N.; Collins, D.; Holford, P.; Milham, P.J. & Conroy, J.P. 2016. Reactions to cadmium stress in a cadmium-tolerant variety of cabbage (*Brassica oleracea* L.): is cadmium tolerance necessarily desirable in food crops? Environmental Science and Pollution Research 23(6): 5296-5306.

Khan, S.; Qureshi, M.I.; Kamaluddin Alam, T. & Abdin, M.Z. 2007. Protocol for isolation of genomic DNA from dry and fresh roots of medicinal plants suitable for RAPD and restriction digestion. African Journal of Biotechnology 6(3): 175-178.

Liu, W.; Yang, Y.S.; Li, P.J.; Zhou, Q.X.; Xie, L.A. & Han, Y.P. 2009. Risk assessment of cadmium-contaminated soil on plant DNA damage using RAPD and physiological indices. Journal of Hazardous Materials 161(2-3): 878-883.

Mishra, V.K. & Tripathi, B.D. 2008. Concurrent removal and accumulation of heavy metals by the three aquatic macrophytes. Bioresource Technology 99(15): 7091-97.

Patra, M.N.; Bhowmik, B. & Bandopadhyay Sharma, A. 2004. Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. Environmental and Experimental Botany 52: 199-223.

Silveira, G.L.; Lima, M.G.F.; Dos Reis, G.B.; Palmieri, M.J. & Andrade-Vieria, L.F. 2017. Toxic effects of environmental pollutants: Comparative investigation using *Allium cepa* L. and *Lactuca sativa* L. Chemosphere 178: 359-367.