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Effect of polyamines and zeolites on the protein profile of leaves of the date palm cuttings *Phoenix dactylifera* L. grown under heavy metal stress conditions

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This study was conducted on the five to six years old offshoots of date palm (Jebjab) cultivar planted in sustainable land, using an environment contaminated with heavy elements, with irrigation water contaminated with heavy elements $Pb(NO_3)_2$ 300 $mg.kg^{-1}$ and cadmium chloride $CdCl_2$ 3 mmol, as well as the comparison treatment. Then the treatment was with some polyamine compounds Putrescine 500 mg L^{-1}, coumaric acid 500 mg L^{-1}, and a zeolite compound at a rate of 10 kg $Palm^{-1}$, in addition to the comparison treatment in the form of ground addition to the soil of the off shoots samples were taken from palm fronds, samples were dried using Freeze-dryer (Lyophilization technique), the protein was extracted from samples, and protein migration was carried out on Polyacrylamide gel using Slab-Electrophoresis method in the presence of SDS denaturants. The molecular weights of proteins were estimated and plotted using PhotoCapt Mw. The results of the study showed that the protein pattern of the leaves show up that all the trees of the study had taken part (identical) with the first protein bundle and the fifth protein bundle on the polyacrylamide gel, as the molecular weights of the proteins of the first bundle ranged between (222.136-243.750) kDa, while the molecular weights of the proteins of the bundle ranged. The fifth is between (24.843-50.386) kDa. This coincidence or participation in the molecular weights of the protein bundles of all the study trees may indicate that these trees belong to one plant origin (which is the Jebjab variety), as these plants share close molecular weights.
Keywords: Date Palm, molecular weights, polyacrylamide gel, polyamine compounds.

INTRODUCTION

The date palm *Phoenix dactylifera. L* belongs to the palm family Arecaceae. This family includes more than 4000 species and nearly 200 genera (Al-Jubouri,2002). The date palm is one of the most important evergreen fruit trees in several countries of the world. Iraq and the Arabian Gulf region are believed to be its original home. It is possible that it originated in southern Iraq. Since ancient times, Iraq was known for palm cultivation, production, and marketing of dates in the world (Bhat AL-Daihan, 2012; Rasheed and AL-Badri, 2018). Date palm trees in Iraq have been subjected to a significant decrease in their production levels and a severe shortage in their numbers during the past two decades (Central Statistical Organization, 2021).

Pollution is defined as any undesirable change in the physical, chemical, or vital properties of the environment (air, water, and soil), which occurs as a result of pollution-causing substances called pollutants, which are any solid, liquid, or gaseous substance found in certain concentrations that are transmitted from different sources and in different quantities (Bhatia, 2009; Al-Wahaibi, 2007). The toxicity of heavy elements has become one of the important and dangerous phenomena and has harmful effects on the morphological form and the decrease in the rate of photosynthesis in the plant due to the closure of stomata as a result of the deposition of elements and the inhibition of enzymatic activities and an imbalance in the water balance as well as affecting the permeability of cell membranes in addition to causing a disturbance in the absorption of nutrients (Kabir *et al.*, 2010). Heavy elements such as lead and cadmium are among the most dangerous elements that pollute soil and water, and the presence of these elements in the soil leads to a decrease in leaf area and a decrease in chlorophyll production, which leads to inhibition in the photosynthesis process and then a decrease in carbohydrates (Singh *et al.*, 2011, Aljaberi, *et al.*,

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2020). And adding cadmium and lead to the soil leads to a significant decrease in protein concentration (Khodaverdiloo *et al.,* 2011, Alhasany, *et al.,* 2020). Exposure of plants to stress of heavy elements is likely to cause phytotoxicity that leads to delayed seed germination, reduced nutrient absorption, disturbances in metabolism, and then weakness in plant growth and development, deterioration of production, and a decrease in the ability to fix molecular nitrogen (Ashraf and Ali, 2007, Alhasany, *et al.*,2021).

Using natural conditioners improves the soil's physical, chemical, and fertility properties, and thus provides a good environment for plant growth in terms of increasing moisture content and providing nutrients, the most important of which is zeolite. The treatment of plants with polyamine compounds such as aspirin, putrescine, and coumaric acid improved the activity of enzymatic antioxidants and the concentrations of non-enzymatic antioxidants, as well as the concentrations of osmotic buffers such as sugars, proline, and others (Yamada *et al.,* 2002).

Due to the increasing rates of heavy metal pollution, these studies have tended to use polyamines to alleviate abiotic stresses such as heavy metal toxicity to maintain normal growth and economic productivity.

MATERIALS AND METHODS

This study was conducted on the five to six years old offshoots of date palm (Jebjab) cultivar planted in sustainable land, using an environment contaminated with heavy elements, with irrigation water contaminated with heavy elements Pb (NO3)2 300 $mg.kg^{-1}$ and cadmium chloride $CdCl_2$ 3 mmol, as well as the comparison treatment. Then the treatment was with some polyamine compounds Putrescine 500 mg L^{-1}, coumaric acid 500 mg L^{-1}, and a zeolite compound at a rate of 10 kg $Palm^{-1}$, in addition to the comparison treatment in the form of ground addition to the soil of the offshoots. Samples were taken from palm fronds and dried by freeze-dryer (lyophilization technique) at a temperature of -26°C. Protein was extracted from the samples according to the method described in (Al-Najjar *et al.,* 2021) by taking 1 g of wicker and placing it in a ceramic mortar with 3 ml of Tris-HCl-buffer (0.1M, pH7.5) containing (PMSF) Phenyl methane sulfonyl fluoride on At a temperature of (4°C), then the centrifugation process was carried out at a temperature of (4°C) and a speed of (18000 R/m) revolution per minute for half an hour, then (40 microliters) of the filtrate was taken to the migration device on a Polyacrylamide gel.

Protein migration was carried out on a polyacrylamide gel using the Slab-Electrophoresis method in the presence of SDS denaturants according to the method described by Bavei et al. The markers were used (Broad Range Protein Molecular Weight Markers) from Promega, and the molecular weights of the proteins were estimated and plotted using a special computer program, PhotoCapt Mw (17version).

The coefficients were numbered on the gel image with the following numbers: Column 1 =marker / Column 2=control / Column 3=cd+put / Column 4=pb+zu / Column 5=pb+co / Column 6=pb+put / Column 7=put / Column 8=cd+zu / Column 9=cd+co / Column 10= pb / Column 11=cd / Column 12=zu / Column 13 = co

RESULTS AND DISCUSSION

From observing the results of the protein pattern of the leaves of the date palm offshoots of the Al-Jibjab cultivar under study (Fig. 2-a-b-c-d-e), we find that there are differences between all the study conditions, as the specifications of the protein bundles differed in terms of size, area, and height according to the treatments which effects in specifications of protein bundles.

The results of the proteolytic pattern of the leaves of the date palm offshoots of the Gijab variety under study (Fig. 3) show that all the studied trees shared (identical) with the first and the fifth protein band on the polyacrylamide gel, as the molecular weights of the first band proteins ranged between (222.136-243.750). kilo Daltons. While the molecular weights of the proteins of the fifth band ranged between (24.843-50.386) kilo Daltons. This coincidence or sharing of the molecular weights of the protein bundles of all the trees of the study may indicate that these trees belong to one plant origin (which is the Jebjab variety), as these trees shared plants with similar molecular weights.

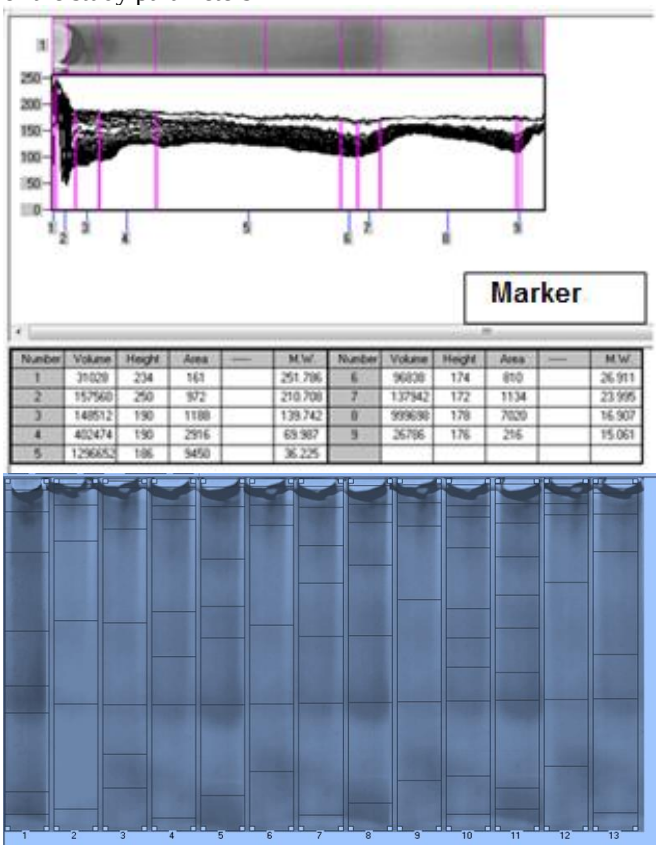
The results of the proteolytic pattern of the leaves of the date palm offshoots of the Gijab variety under study (Fig. 3) show that there are differences between these trees in the number, locations, and specifications of the protein bundles on the polyacrylamide gel. The number of protein bundles ranged between (6-10) protein bundles depending on the Treatment type, it was six protein bundles in each of the overlapping treatment (lead with putrescine) and the overlapping treatment (cadmium with coumaric acid), while it appeared in most of the treatments (comparison, cadmium with putrescine, lead with zeolite, lead with coumaric acid, tyrosine, cadmium With zeolite, zeolite, and coumaric acid) seven protein bundles, as when observing these treatments (treatment with bitcoin, coumaric acid, and zeolite and their interactions with pollution treatments), we find that these compounds (pollution treatments) when they interfered with lead and cadmium prevented the change of the protein pattern of trees, as the number The routine packets have an equal number of protein packets for the comparison treatment.

It is noted from the migration results of the proteins on the polyacrylamide gel that there are ten protein bundles in each of the lead contamination treatment and the cadmium contamination treatment, with closeness or conformity in the molecular weights of the proteins in each of those bundles for both treatments.

Also the study treatments had a clear effect on the gene expression process of the trees and caused the emergence of new protein bundles that reached ten bundles (as in the treatment of lead and cadmium contamination), as this indicates that the study treatments have stimulated the cells to manufacture new proteins that support Indicators of growth and development of growing plants under the stress of pollution with heavy elements. The treatments also had a clear effect in changing the locations of protein packages and their different molecular weights, which indicates that the treatments have caused activation of the gene expression process and the manufacture of new proteins that may have a role in improving plant growth and resistance to pollution.

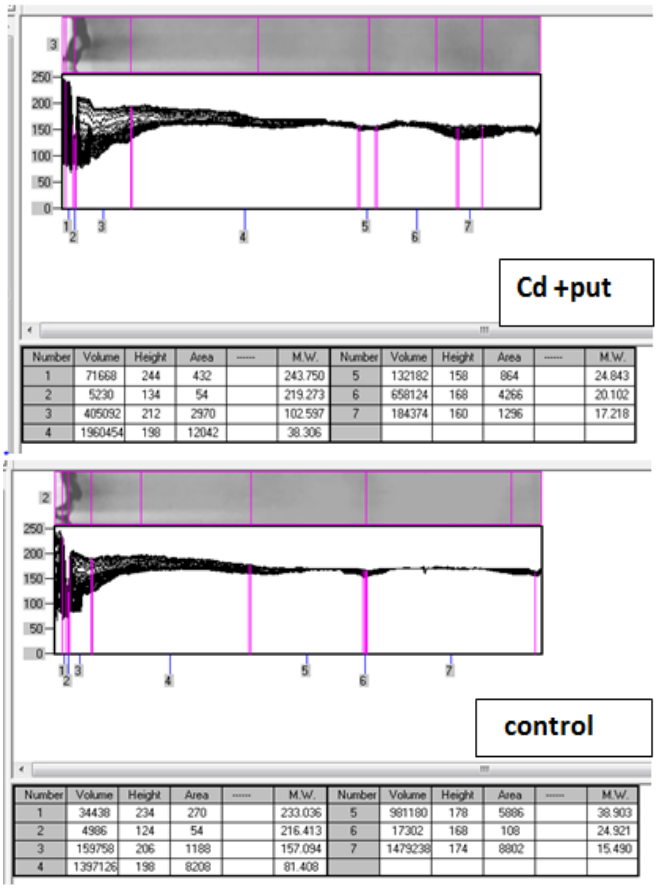
These results indicate that treating plants with pollutants may lead to the synthesis of natural proteins, as well as a change in the translation and transcription processes, which leads to the production of new proteins through the gene expression process according to the plant's need and in response to the type of treatment to ensure the improvement of plant growth (David and Nilson. 2000 ; Khairallah, 2009).

Figure (1): The polyacrylamide gel shows the protein bands of the study parameters



Number	Volume	Height	Area	-----	M.W.	Number	Volume	Height	Area	-----	M.W.
1	31029	234	161		251.786	6	96838	174	810		26.911
2	157560	250	972		210.708	7	137942	172	1134		23.995
3	148512	190	1188		139.742	8	999698	178	7020		16.907
4	402474	190	2916		69.987	9	35786	176	216		15.061
5	1296652	186	9450		36.225						

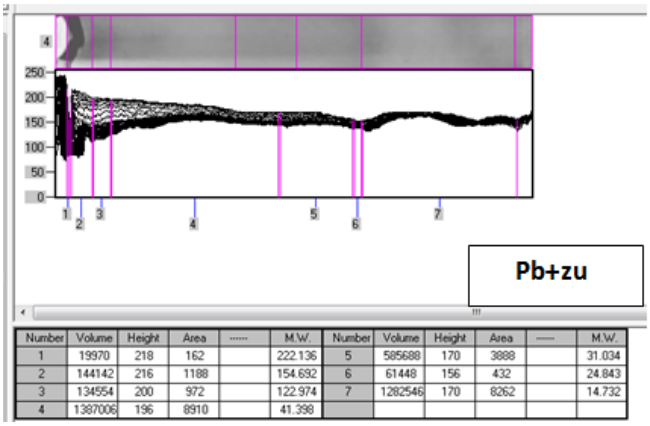
Figure (2-a). Some specifications of the protein bands on the polyacrylamide gel for the marker



Number	Volume	Height	Area	-----	M.W.	Number	Volume	Height	Area	-----	M.W.
1	71668	244	432		243.750	5	132182	158	864		24.843
2	5230	134	54		219.273	6	658124	168	4266		20.102
3	405092	212	2970		102.597	7	184374	160	1296		17.218
4	1960454	198	12042		38.306						

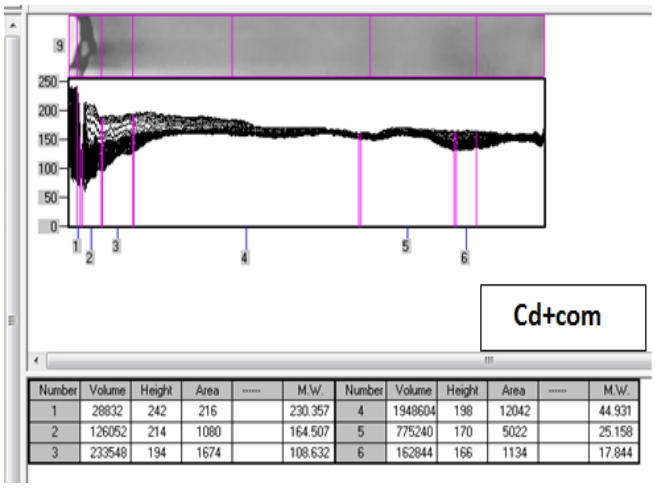
Number	Volume	Height	Area	-----	M.W.	Number	Volume	Height	Area	-----	M.W.
1	34438	234	270		233.036	5	981180	178	5886		38.903
2	4986	124	54		216.413	6	17302	168	108		24.921
3	159758	206	1188		157.094	7	1479238	174	8802		15.490
4	1397126	198	8208		81.408						

Figure (2-b). Some specifications of protein bands on polyacrylamide gel

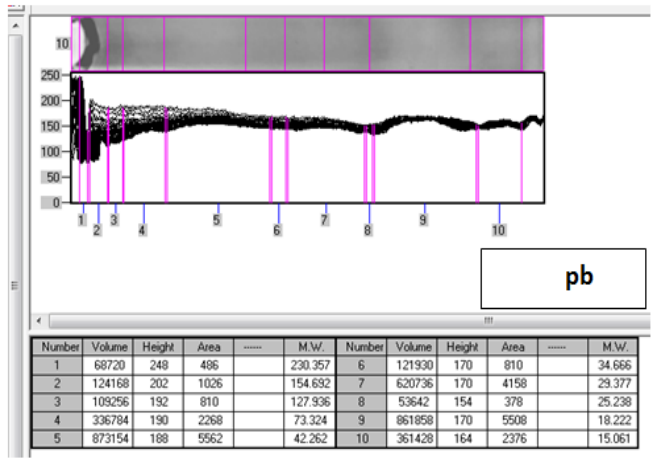


Number	Volume	Height	Area	-----	M.W.	Number	Volume	Height	Area	-----	M.W.
1	19970	218	162		222.136	5	585688	170	3888		31.034
2	144142	216	1188		154.692	6	61448	156	432		24.843
3	134554	200	972		122.974	7	1282546	170	8262		14.732
4	1387006	196	8910		41.398						

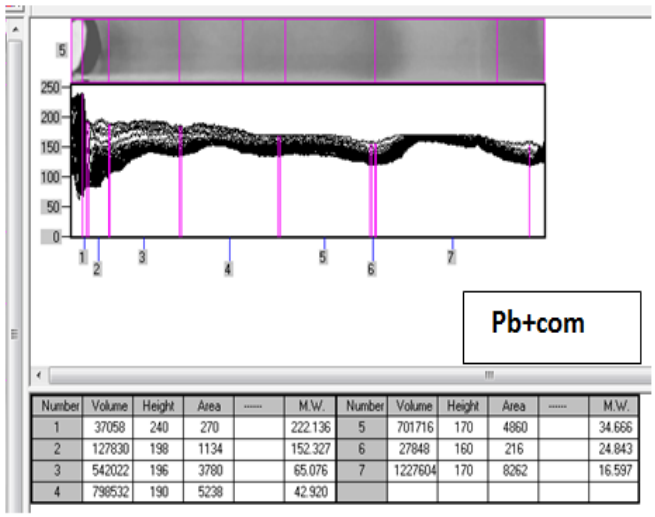
Figure (2-b). Some specifications of protein bands on polyacrylamide gel



Number	Volume	Height	Area	.....	M.W.	Number	Volume	Height	Area	.....	M.W.
1	28832	242	216		230.357	4	1948604	198	12042		44.931
2	126052	214	1080		164.507	5	775240	170	5022		25.158
3	233548	194	1674		108.632	6	162844	166	1134		17.844

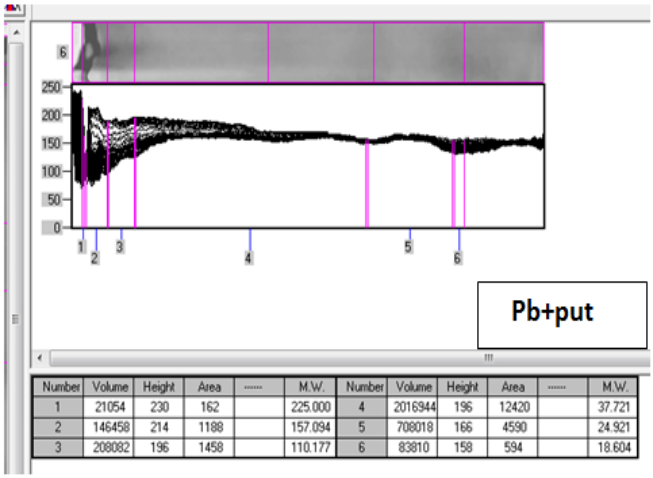


Number	Volume	Height	Area	.....	M.W.	Number	Volume	Height	Area	.....	M.W.
1	68720	248	486		230.357	6	121930	170	810		34.666
2	124168	202	1026		154.692	7	620736	170	4158		29.377
3	109256	192	810		127.936	8	53642	154	378		25.238
4	336784	190	2268		73.324	9	861858	170	5508		18.222
5	873154	188	5562		42.262	10	361428	164	2376		15.061

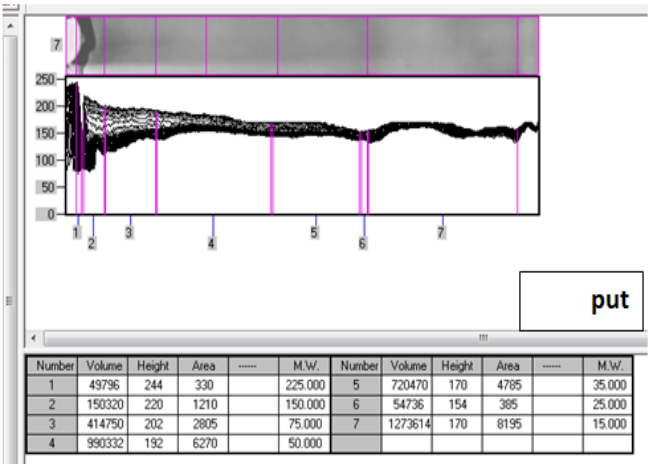


Number	Volume	Height	Area	.....	M.W.	Number	Volume	Height	Area	.....	M.W.
1	37058	240	270		222.136	5	701716	170	4860		34.666
2	127830	198	1134		152.327	6	27848	160	216		24.843
3	542022	196	3780		65.076	7	1227604	170	8262		16.597
4	798532	190	5238		42.920						

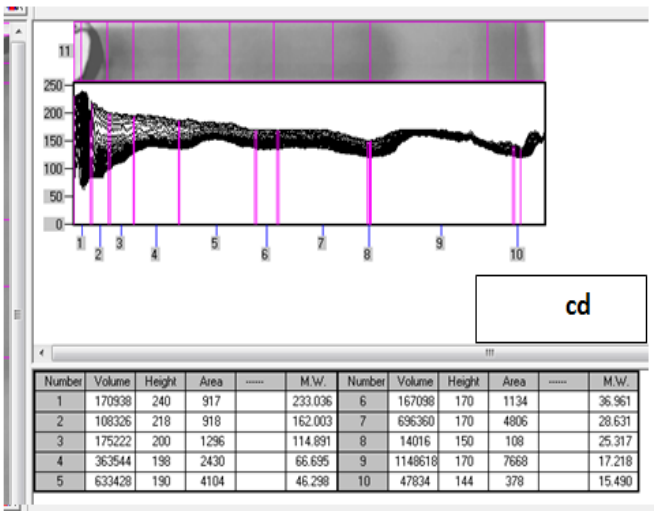
Figure (2-c). Some specifications of protein bands on polyacrylamide gel



Number	Volume	Height	Area	.....	M.W.	Number	Volume	Height	Area	.....	M.W.
1	21054	230	162		225.000	4	2016944	196	12420		37.721
2	146458	214	1188		157.094	5	708018	166	4590		24.921
3	208082	196	1458		110.177	6	83810	158	594		18.604

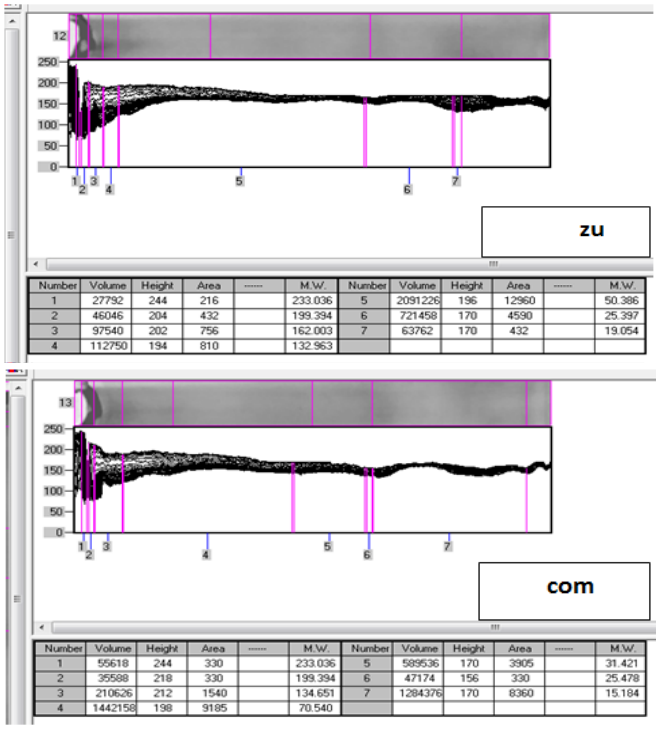


Number	Volume	Height	Area	.....	M.W.	Number	Volume	Height	Area	.....	M.W.
1	49796	244	330		225.000	5	720470	170	4785		35.000
2	150320	220	1210		150.000	6	54736	154	385		25.000
3	414750	202	2805		75.000	7	1273614	170	8195		15.000
4	990332	192	6270		50.000						



Number	Volume	Height	Area	.....	M.W.	Number	Volume	Height	Area	.....	M.W.
1	170938	240	917		233.036	6	167098	170	1134		36.961
2	108326	218	918		162.003	7	696360	170	4806		28.631
3	175222	200	1296		114.891	8	14016	150	108		25.317
4	363544	198	2430		66.695	9	1148618	170	7668		17.218
5	633428	190	4104		46.298	10	47834	144	378		15.490

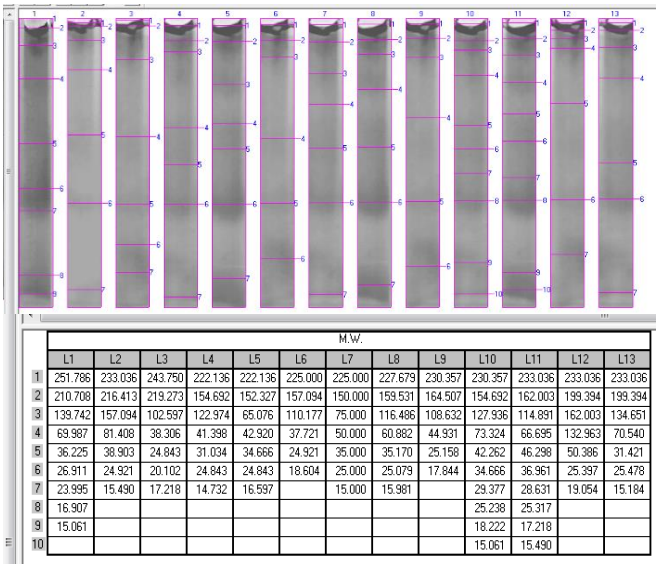
Figure (2-d). Some specifications of protein bands on polyacrylamide gel



Number	Volume	Height	Area	.....	M.W.	Number	Volume	Height	Area	.....	M.W.
1	27792	244	216		233.036	5	2091226	196	12960		50.386
2	46046	204	432		199.394	6	721458	170	4590		25.397
3	97540	202	756		162.003	7	63762	170	432		19.054
4	112750	194	810		132.963						

Number	Volume	Height	Area	.....	M.W.	Number	Volume	Height	Area	.....	M.W.
1	55618	244	330		233.036	5	589536	170	3905		31.421
2	35588	218	330		199.394	6	47174	156	330		25.478
3	210626	212	1540		134.651	7	1284376	170	8360		15.184
4	1442158	198	9185		70.540						

Figure (2-e) Some specifications of protein bands on polyacrylamide gel



	M.W.												
	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13
1	251.786	233.036	243.750	222.136	222.136	225.000	225.000	227.679	230.357	230.357	233.036	233.036	233.036
2	210.708	216.413	219.273	154.692	152.327	157.094	150.000	159.531	164.507	154.692	162.003	199.394	199.394
3	139.742	157.094	102.597	122.974	65.076	110.177	75.000	116.486	108.632	127.936	114.891	162.003	134.651
4	69.987	81.408	38.306	41.398	42.920	37.721	50.000	60.882	44.931	73.324	66.695	132.963	70.540
5	36.225	38.903	24.843	31.034	34.666	24.921	35.000	35.170	25.158	42.262	46.298	50.386	31.421
6	26.911	24.921	20.102	24.843	24.843	18.604	25.000	25.079	17.844	34.666	36.961	25.397	25.478
7	23.995	15.490	17.218	14.732	16.597		15.000	15.981		29.377	28.631	19.054	15.184
8	16.907									25.238	25.317		
9	15.061									18.222	17.218		
10										15.061	15.490		

Figure 3. The number and locations of protein packages and their molecular weights (a side of the Photocapt program). Column 1 =marker / Column 2=control / Column 3=cd+put / Column 4=pb+zu / Column 5=pb+co / Column 6=pb+put / Column 7=put / Column 8=cd+zu / Column 9=cd+co / Column 10= pb / Column 11=cd / Column 12=zu / Column 13 = co

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***Ethical statement*:** This article does not contain any studies with human participants or animal performed by any of the authors.

***Availability of data and material*:** We declare that the submitted manuscript is our work, which has not been published before and is not currently being considered for publication elsewhere .

***Code Availability*:** Not applicable

***Consent to participate*:** All authors are participating in this research study.

***Consent for publication*:** All authors are giving the consent to publish this research article in JGIAS

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