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Tests for inhibiting the action of cadmium by selective interactions with Zinc and Copper; In-Vitro investigations on the Phaseolus-Vulgaris plant.

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Abstract— Knowing that heavy metals are characterized by the cumulative effects on the one hand and that they are present in biological systems at very low doses, which makes their effects latent and often irreversible. It is in this perspective and in order to try to solve the problem of detoxification of this type of major contaminant, we opted for an effective solution which is detoxification by antagonism based on selective interaction. The choice is made for Cadmium which is considered a very harmful element on the one hand and its almost total presence in the entire ecosystem. The investigation protocol is based on two complementary steps which are:

• Determination of the Cadmium toxicity threshold vis-à-vis the selected plant Phaseolus-Vulgaris.

• Study of the interaction between and Cadmium and Zinc and Cadmium Copper selected for the application

And the verification of the reduction of the harmful effect by selective competition.

Keywords— Threshold of toxicity, Cadmium, Investigations, Phaseolus-vulgaris, Interaction, Zinc, Copper, Antagonism.

I. INTRODUCTION

In recent years the use of chemicals has grown rapidly in almost every area of our lives.

Pollution therefore appears as one of the crucial problems of modern civilization, the accelerated and often disorderly development of industry is one of the main causes of pollution. Among the sources of pollution, we can cite heavy metal contamination which affects all ecosystems, namely atmospheric pollution, microbiological pollution, soil pollution and finally water pollution even threatening our food.

It should be noted that even if some of these metals have a vital biological role, the fact remains that they become very toxic in high concentrations such as copper zinc iron etc. ....

Taking into account the seriousness that this type of toxicant can cause on the food chain by accumulation mechanism, we opted for an original study proposal, namely the tests to minimize the effects of toxic Cadmium preselected by in vitro investigations carried out. on the plant (Phaseolus-vulgaris) and this by researching the antagonistic effect by selective Cadmium / Zinc interaction on the one hand and Cadmium / Copper interaction on the other hand in order to optimize the quality of the effect. Taking into account the seriousness that this type of toxicant can cause on the food chain by accumulation mechanism, we opted for an original study proposal, namely the tests to minimize the effects of toxic Cadmium preselected by in vitro investigations carried out. on the plant (Phaseolus-vulgaris) and this by researching the antagonistic effect by selective Cadmium / Zinc interaction on the one hand and Cadmium / Copper interaction on the other hand in order to optimize the quality of the effect.

the choice is made on Cadmium because this element has no function to fulfill in biological organisms [12] in addition taking into account its contamination of our ecosystem because the emissions are without prior treatment, on the other hand its gradual accumulation by low doses can induce harmful effects on the plant system in particular and the food chain in general. Cadmium induces an inhibition of the growth of different parts of plants. Thus, it produces a reduction in the biomass of different organs in plants also such as beans [35]. Long-term exposure to cadmium produces in the leaves, the appearance of the phenomenon of chlorosis due to a decrease in the chlorophyll content [3]. Cadmium, is a highly reactive metal which stimulates the formation of ROS, leading to the development of secondary oxidative stress [22].

The first stage of the investigation consists in carrying out two additional tests in order to determine the minimum dose of the toxicant (Cadmium) which can cause the appearance of the first symptoms of toxicity.

The second stage of the investigation is the minimization test of the toxic effects of Cadmium by the selective interaction Cadmium / Zinc and Cadmium / Copper selected because they are oligo-elements which have a biological role in biological systems and to look for which one. of the two elements gives satisfactory results in terms of antagonism on the Phaseolus-vulgaris plant chosen for our investigation.

Thus, the investigation protocol follows the following steps:
1- Determination of the minimum concentration of Cadmium that can generate harmful effects.
2- Tests to minimize the harmful effects of Cadmium by the mechanism of interaction with a desirables element which are Zinc and Copper.

Protocol of study:

I. Determination of toxicity threshold of Cadmium:

Introduction:
The purpose of experiments 1 and 2 is to determine the toxicity threshold, or the minimum concentration of Cadmium capable of causing the appearance of symptoms of toxicity.
To determine this concentration, we opted first by injecting the high concentrations, then we started to decrease as the symptoms persist.
From the visual observations, the range of concentrations from 1.0 to 0.008 ppm was retained.

A. Empirical protocol:

A.1.1 Means of experimentation:

At the end of detecting the actions, the plants are used as a bio indicator of pollution by trace metals, since they quickly exhibit toxicity: Chlorosis, necrosis etc. ...
As well as the specific accumulation of trace metals in the tissues of test plants results from a sample or absorption, as well as the mechanism of metabolism and internal transport.
The accumulation is calculated according to the transport of the trace metal content of the cultivated species, and the control plant not exposed to the toxic. It is for these reasons that our choice is made for one of the most sensitive varieties (Phaseolus-vulgaris), which is an annual plant of the legume family with fast vegetation, whose stem is thin, medium leaves, it has the advantages during the experimentation period.
1- Its incidence and the real effects of the element on the living organism.
2- The calculation of compensation for damage to crops.
3- The limited duration of germination.
4- The appearance of toxic symptoms and rapid and visible.

A.1.2 Cultivation of plants:

In order to follow the evolution of the toxic, cultures of (Phaseolus-Vulgaris) are carried out.

A.1.2.1 Cultivation mode:

The plants are cultivated in a culture chamber designed for this purpose, whose intrinsic and extrinsic factors are respected.
• Intrinsic factors: Temperature is around 25º, adequate light; 16 hours of light and 8 hours of darkness to ensure good photosynthesis.
• Extrinsic factors: The seeds in good condition are sown in neutral soil, namely pH = 7, a cation exchange capacity (CEC = 0); plants are watered with distilled water to avoid possible interference.

A.1.2.2 the duration:

Once germination is accomplished through the use of its own stores for a period of seven days. The plants need other reserves to survive, after seven days the plants are transferred to a rich medium which is the nutrient solution.

A.1.2.3: Transfer:

The transfer consists in taking the young seedlings and placing them in a nutritive solution which ensures the normal growth of the plant, and to prepare them for the investigation.

This solution consists mainly of the following elements:

Elements	g/l (Distilled water)
HBO_3	0.05
NaCl	0.25
$Ca\,(NO_3).\,4\,H_2O$	0.49
KNO_3	1.00
$Mg\,SO_4.\,7\,H_2O$	0.50
$Fe\,SO_4\,.7\,H_2O$	0.05
$Cu\,SO_4.\,5\,H_2O$	0.05

B. Final Stage

B.1.1 Method of sample preparation:

Before starting the analysis, a preliminary treatment is necessary, this treatment consists of wet mineralization or digestion, the aim of which is to remove organic matter to avoid chemical interference through the nitric acid / acid combination perchloric.
• Plant size measurement and fresh weight
• Cutting plants into small parts
• Dryer in an oven at a temperature varying from 120ºC to 140ºC. For 8-10 hours.
• Crushing the plants in mortar, measuring the dry weight, which is put in a 100 ml beaker, adding 10 ml of nitric acid and the same perchloric acid in addition to EDTA.
• Heating on hot plate at a temperature of 120ºC.
• Once the digestion is complete, the solution becomes more or less clear with evaporation of the excess acid.
• The solution is filtered, so the samples are ready for analysis.

C. Material and methods:

For the estimation of toxic concentrations, the choice is made for atomic absorption spectroscopy, because it has several advantages such as: selectivity, universality, speed of execution, high sensitivity and cheaper cost of analysis.
This technique is on the beer-lambert law, expressed by the unique relation:
$\text{Log } P / P0 = e.\, b\, .c = A$
$P / P0$: Radiation energies in erg / cm^2.
A: Absorbance
B: Opening distance of the burner
C: Concentration of the element (mol/l)
E: Coefficient of molar absorbency.

II. DETERMINATION OF THRESHOLD TOXICITY OF CADMIUM:

The aim of experiments 1 and 2 is to determine the threshold for the toxicity of Cadmium (Cd^{2+}) capable of causing the appearance of the first symptoms of toxicity; and to achieve this objective the choice is brought to a wide range of concentrations.

A.: Test 1: Determination of the toxicity threshold of Cadmium:

Samples [Cd] ppm	D1	D2	D3	D4	D5	D6	D7
W.pt	-	-	-	-	-	-	-
S1 (1.0)	SL	SL	SL+BC	SL+BC	SL+BC+BN	SL+BC+BN	SL+BC+BN
S2 (0.08)	-	SL	SL+BC+BN	SL+BC+BN	SL+BC+BN	SL+BC+BN	SL+BC+BN
S3 (0.06)	-	SL	SL+BN	SL+BC+BN	SL+BC+BN	SL+BC+BN	SL+BC+BN
S4 (0.04)	-	-	SL	SL	SL+BC+BN	SL+BC+BN	SL+BC+BN
S5 (0.02)	-	-	-	-	SL+BC+BN	SL+BC+BN	SL+BC+BN
S6 (0.01)	-	-	-	-	-	BC	BC+BN
S7 (0.009)	-	-	-	-	-	-	-

Table 1: Visual observations Test 1

SL: Shrinking leaves **C:** Chlorosis **BC**: Beginning of chlorosis **BN**: Beginning of necrosis
W.pt: Witness plant.

Samples [Cd] ppm	S. Pt (Cm) B. F	S. Pt (Cm) A. F	F.W Pt (g) B.t	F.W Pt (g) A.t	D.W Pt(g)	NS. V (mi) A.t
W.pt	14.50	15.00	1.26	1.81	0.14	73
S1 (0.06)	15.50	16.00	1.66	2.00	0.18	73
S2 (0.04)	17.00	17.80	1.36	2.04	0.12	71
S3 (0.02)	18.00	19.00	1.80	2.02	0.16	73
S4 (0.01)	18.00	18.50	1.70	2.01	0.16	72

Table 2: plant sizes before and after transfer with fresh and dry weight of plants and volume of nutrient solution Test 1

S. Pt B F: Size of plant before filtration **S. Pt A F**: Size of plant after filtration **Sp. Bt** : Size of the plant before transfer **Sp. At**: Size of the plant after transfer **FWp.Bt**: Fresh weight of the plant before transfer **FWp.At**: Fresh weight of the plant after transfer **DWp**: Dry weight of the plant **NS. V**: Nutrient solution volume **CHL**: Chlorosis.

Samples [Cd] ppm	Abs (Pt)	Abs (SN)	[Cd] Pt ppm	[Cd] SN ppm	% R Recovery
W.pt	-	-	-	-	-
S1 (0,009)	0.025	0.006	0.002	0.003	74
S2 (0,01))	0.049	0.071	0,1	0,21	93
S3 (0.02)	0.062	0.114	0,25	0,56	84
S4 (0,04)	0.066	0.154	0,42	0,85	77
S4 (0,06)	0,072	0,164	0,54	1,26	79

Table 3: Cadmium concentrations determined by AAS Test 1

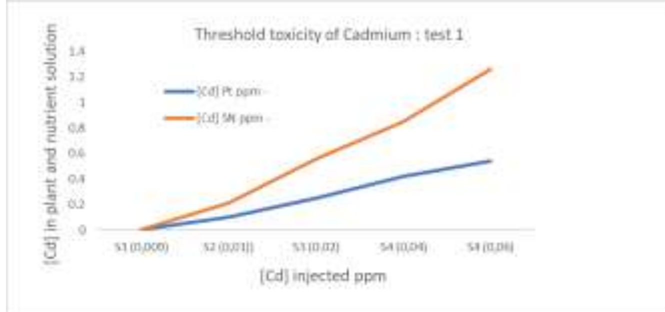

Fig1: Test 1: Determination of the toxicity threshold of Cadmium

B: Test 2 Determination of the toxicity threshold of Cadmium:

	D1	D2	D3	D4	D5	D6	D7
W.pt	-	-	-	-	-	-	-
S1 (1.0)	-	SL	SL	SL+BN	SL+BN	SL+BN	SL+BN
S2 (0.08)	-	-	SL+BN	SL+BN	SL+BN	SL+BN	SL+BN
S3 (0.06)	-	SL	SL	RF+DN	SL+BN	RF	SL+BN
S4 (0.04)	-	-	SL	SL	SL+BN	RF+DN	SL+BN
S5 (0.02)	-	-	-	-	SL	SL	SL+BN
S6 (0.01)	-	-	-	-	-	SL	SL+BN
S7(0.009)	--	-	-	-	-	-	-
S8(0.008)	-	-	-	-	-	-	-

Table 4: Visual observations Test 2

Samples [Cd] ppm	S. Pt (Cm) B. F	S. Pt (Cm) A. F	F.W Pt (g) B.t	F.W Pt (g) A.t	D.W Pt(g)	NS. V (mi) A.t
W.pt	11.00	11.40	2.27	2.86	0.14	69
S1 (0.01)	11.50	11.50	1.67	2.01	0.16	77
S2 (0.02)	10.00	11.20	1.48	1.95	0.16	76
S3 (0.04)	13.00	14.50	2.12	2.58	0.12	73
S4 (0.06)	10.00	11.00	1.40	1.76	0.18	72

Table 5: plant sizes before and after transfer with fresh and dry weight of plants and volume of nutrient solution Test 2.

Samples [Cd] ppm	Abs (Pt)	Abs (SN)	[Cd] Pt ppm	Cd] SN ppm	% R Recovery
W.pt	-	-	-	-	-
S1 (0,009))	0.002	0.003	0.001	0.002	78
S2 (0,01))	0.042	0.067	0,12	0,28	91
S3 (0.02)	0.053	0.110	0,21	0,46	74
S4 (0,04)	0.061	0.150	0,39	0,77	77
S5 (0,06)	0.077	0.041	0,48	1,05	78

Table 6: Cadmium concentrations determined by AA Test 2

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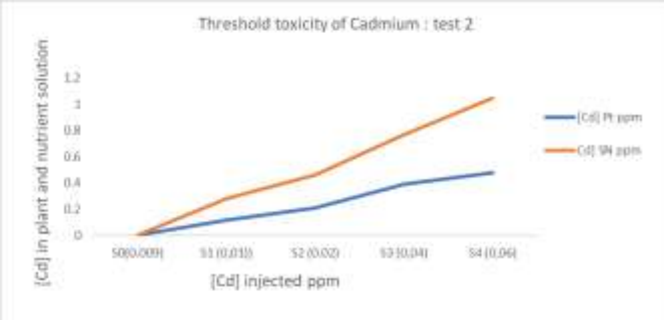

Fig 2: Test 2: Determination of the toxicity threshold of Cadmium

C. Discussion of results:

The first step in the investigation is to determine the minimum concentration that can cause the development of biological systems.

Therefore, we opted for a massive culture of batches of plants in the culture chamber, and we proceeded to injections of decreasing doses per batch of 10 plants, the first results showed us that at high doses the plants are wilted on the first and second day.

Arrived at low doses, the investigation begins to give weekly follow-ups with evolution of symptoms of toxicity, until the selection of the study ranges from 1 to 0.008 ppm.

The follow-up by visual observations showed us through the two tests and by verification of the effects by the witnesses, that the minimum dose inducing the first signs of toxicity is 0.01 ppm, result which converges with the work of [19].[1].

The confirmation of the results is verified by the findings on the control plant and consolidated by the empirical results by AAS.

Referring to the results tables 1-2-3; Fig 1 Test 1 and Tables 4-5-6 and Fig 2 Test 2, we notice that the concentration is proportional to the absorbance, so we can deduce that the plants exposed to this range of Cadmium concentrations is admire from 1-40 ppm are predisposed to absorb the toxic and that translates into the results obtained.

Beyond the 0.01 ppm concentration of Cadmium injected, it can be seen that the amount of Cadmium retained in the nutrient solution is greater than the amount of Cadmium absorbed by the plant, the accumulation of cadmium is greater in the leaves than in the roots. These results are in agreement with the results of [10] and [12], this is explained by the saturation of plants exposed to the poison: this saturation can be explained by the effect of Cadmium on the metabolism of plants so that these become chlorotic. This loss of chlorophyll is caused by the reduction in plant photosynthesis. As well as Cadmium acts as an inhibitor of enzymatic activity by neutralizing the S-H group of metallothionine necessary for the catalytic activity where it substitutes other divalent cations in the metallos enzymes interfere with the transport of electrons in chloroplasts [26].[24].

All these criteria will lead to exclusion phenomena, finally we can conclude from the results obtained that the threshold of toxicity of Cadmium vis-à-vis the plant Phaseolus-Vulgaris exposed is estimated at 0.01 ppm.

This result is consolidated by the visual observations which enabled us to follow the behavior of the plants vis-à-vis the toxic (Cadmium) injected during a period of seven days (see Table 1 Test1, table 4 Test 2).

Regarding the percentage recovery of Cadmium; we notice that we have a very good rotation of the results, because the percentage varies between 70 and 95%, since the plants selected for the investigation are of acceptable uniformity from the point of view of size and weight (see table 2 Test 1 and Table 5 Test 2), in order to consolidate our practical results, we opted to verify the error rate by developing standard deviations which verified the validity of our results.

The results are consolidated by the empiric analyzes by AAS Tales 3 Test 1 and Table 6 Test 2. The validity of the results is confirmed by visual observations and concentrations by spectroscopic analyzes on the control plant.

It can be concluded for this first phase of the investigation that cadmium is one of the most toxic trace elements towards biological systems and this is mainly due to the fact that Cd penetrates more easily into the leaf than Pb which is mainly adsorbed to epicuticular lipids at the surface [15].[2]

The two iterative tests confirm to us that the low dose inducing the first signs of toxicity is deduced by visual observation and the AAS analysis shows the severity of this toxic with respect to the bioindicator selected for our investigation, work which agrees with the conclusions of [29] .

III. INTERACTION TESTS:

A. Cadmium/Zinc tests:

To carry out this in-vitro investigation, the choice of the first element retained is Zinc because it has several advantages such as bringing together the physicochemical properties with those of cadmium, in addition it is a desirable element for all biological organisms.

The first step is focused on the simultaneous injection of Zinc at variable doses with the constant cadmium dose of 0.01 ppm, after several tests the doses ranging from 1 to 40 ppm were selected.

A.1.1 Test 1: Cadmium / Zinc interaction:

Fixed concentration Cd: 0.01 Variable concentrations Zinc: 1-5-10-15-25-30-40 ppm

Samples Cd ppm	D1	D2	D3	D4	D5	D6	D7
W.pt	-	-	-	-	-	-	-
S1 (1-0.01)	-	-	SL+ CHL	SL+ CHL	SL+ CHL	SL+ CHL	SL+ CHL
S2 (5-0.01)	-	-	SL	SL+ CHL	SL+CHL	SL+ CHL	SL+ CHL
S3 (10-0.01)	-	-	SL	SL	SL	SL	SL
S4 (15-0.01)	-	-	SL	SL	SL	SL	SL
S5 (25-0.01)	-	-	-	SL	SL	SL	SL
S6 (30-0.01)	-	-	-	-	-	-	-

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S7 (40-0.01)	--	-	-	-	-	-	-

Table 7: Visual observations Test 1

Samples [Zn]/[Pb]/ ppm	S. Pt (Cm) B. F	S. Pt (Cm) A. F	F.W Pt (g) B.t	F.W Pt (g) A.t	D.W Pt(g)	NS. V (ml) A.t
W.pt	14.50	15.0	1.89	2.27	0.17	60
S1 (1-0.01)	13.5	14.0	1.79	2.11	0.14	73
S2 (5-0.01)	12.5	13.25	1.76	2.05	0.14	74
S3 (10-0.01)	11.5	12.0	2.21	2.50	0.16	73
S4 (15-0.01)	12.0	12.90	1.81	2.24	0.16	75
S5 (25-0.01)	11.0	11.80	1.93	2.20	0.17	76
S6 (30-0.01)	12.5	13.25	1.95	2.35	0.18	76
S7 (40-0.01)	12.0	13.0	1.99	2.40	0.18	76

Table 8: size of plants before and after transfer with fresh and dry weight of plants and volume of nutrient solution Test 1

Samples [Zn]/[Cd]/ ppm	Abs (Pt)	Abs (SN)	[Cd] Pt ppm	[Cd] SN ppm	% Recovery
W.pt	0.002	0.003	0.001	0.002	-
S1 (1-0.01)	0.100	0.024	0,58	0,12	95
S2 (5-0.01)	0.096	0.030	0,46	0,24	97
S3 (10-0.01)	0.090	0.036	0,35	0,39	92
S4 (15-0.01)	0.088	0.039	0,21	0,45	94
S5 (25-0.01)	0.078	0.043	0,15	0,56	92
S6 (30-0.01)	0.068	0.058	0,10	0,65	98
S7(40-0.01)	0.057	0.069	0,05	0,74	97

Table 9: Cadmium concentrations determined by AAS Test 1

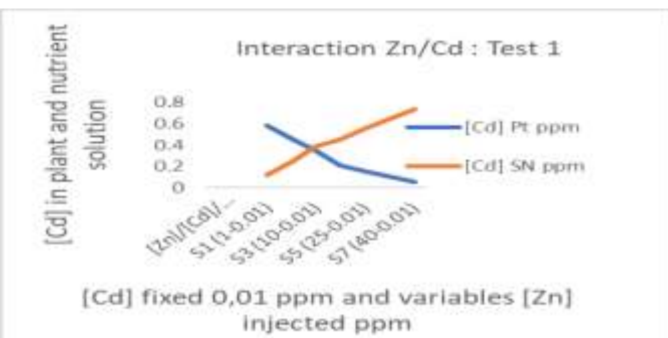

Fig 3: Interaction Cd/Zn: Test 1

	Plante			Solution nutritive		
[Cd]/ [Zn] ppm	X	SD	X+2SD	X	SD	X+2SD
W.pt	0.003	0.0033	0.003+ 0.0066	0.03	0.0027	0.003+ 0.0054
S1 (1-0.01)	0.045	0.0057	0.045+ 0.0114	0.096	0.0076	0.0096+0. 00114
S2 (5-0.01)	0.051	0.0025	0.051+ 0.0050	0.079	0.0035	0.079+ 0.0070
S3 (10-0.01)	0.046	0.0027	0.046+ 0.0054	0.065	0.0035	0.065+ 0.0070
S4 (15-0.01)	0.045	0.0047	0.045+ 0.0094	-	-	-
S5 (25-0.01)	0.044	0.0037	0.044+ 0.0074	0.058	0.0045	0.058+ 0.0090
S6 (30-0.01)	0.042	0.0030	0.042+ 0.0060	0.047	0.0030	0.047+ 0.0060
S7(40-0.01)	0.040	0.0027	0.042+ 0.0054	0.032	0.0026	0.32+ 0.0052

Tableau 10: Estimated values of standard deviation Test 1

A.1.2 Test 2: Cadmium / Zinc interaction:

Cadmium / Zinc interaction Fixed concentration Cd: 0.01 ppm

Variable concentrations Zinc: 1-5-10-15-25-30-40 ppm

Samples [Zn]/[Cd]/ ppm	D1	D2	D3	D4	D5	D6	D7
W.pt	-	-	-	-	-	-	-
S1 (1-0.01)	-	-	-	-	-	-	-
S2 (5-0.01)	-	-	SL	SL	SL	SL	SL
S3 (10-0.01)	-	-	SL	SL+BC	SL+BC	SL+BC	SL+BC
S4 (15-0.01)	-	-	SL	SL+BC	SL+BC	SL+BC	SL+BC
S5 (25-0.01)	-	-	-	SL+BC	SL+BC	SL+BC	SL+BC
S6 (30-0.01)	-	-	-	-	-	-	BC
S7(40-0.01)	--	-	-	-	-	-	-

Table 11: Visual observations Test 2

Samples [Zn]/[Pb]/ ppm	S. Pt (Cm) B. F	S. Pt (Cm) A. F	F.W Pt (g) B.t	F.W Pt (g) A.t	D.W Pt(g)	NS. V (mi) A.t
W.pt	14.50	15.0	1.80	2.17	0.15	62
S1 (1-0.01)	13.0	13.80	1.68	2.08	0.12	72
S2 (5-0.01)	13.25	14.0	1.68	2.02	0.14	74
S3 (10-0.01)	12.0	12.68	2.17	2.61	0.15	74
S4 (15-0.01)	12.5	13.01	1.82	2.21	0.14	76
S5 (25-0.01)	11.25	11.48	1.88	2.19	0.18	75
S6 (30-0.01)	12.75	13.50	1.97	2.41	0.19	77
S7 (40-0.01)	12.0	12.80	1.95	2.38	0.19	77

Table 12: sizes of plants before and after transfer with fresh and dr

Samples [Zn]/[Cd] ppm	Abs (Pt)	Abs (SN)	[Cd] Pt ppm	[Cd] SN ppm	% Recovery
W.pt	0.002	0.002	0.001	0.002	-
S1 (1-0.01)	0.099	0.021	0,65	0,16	95
S2 (5-0.01)	0.091	0.032	0,51	0,25	96
S3 (10-0.01)	0.086	0.037	0,34	0,38	99
S4 (15-0.01)	0.075	0.041	0,28	0,40	92
S5 (25-0.01)	0.070	0.044	0,20	0,56	94
S6 (30-0.01)	0.064	0.053	0,16	0,65	97
S7 (40-0.01)	0.053	0.072	0,10	0,79	96

Table 13: Concentrations determined by AAS Test 2

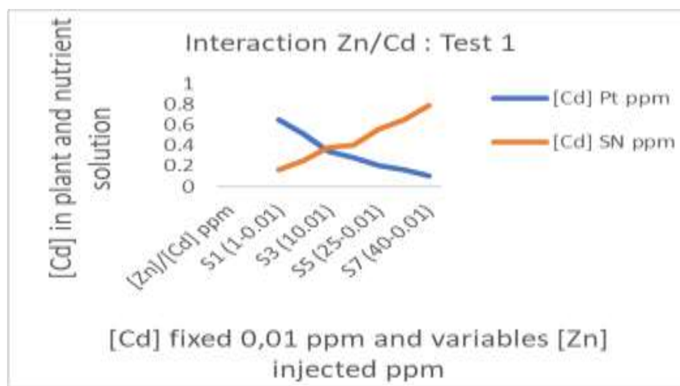

Fig 4: Interaction Cd/Zn: Test 2

	Plant			Nutrient solution		
Samples [Zn]/[Pb] ppm	X	SD	X+2SD	X	SD	X+2SD
W.pt	-	-		-	-	-
S1 (1-0.01)	0.046	0.0058	0.046+0.0116	0.095	0.0075	0.0096+0.015
S2 (5-0.01)	0.050	0.0025	0.050+0.0050	0.078	0.0036	0.079+0.072
S3 (10-0.01)	0.044	0.0025	0.044+0.0050	0.064	0.0035	0.064+0.0070
S4 (15-0.01)	0.045	0.0048	0.045+0.0096	0.077	0.0023	0.077+0.0046

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S5 (25-0.01)	0.044	0.0038	0.044+0.0076	0.056	0.0046	0.056+0.0092
S6 (30-0.01)	0.044	0.0032	0.04440.0064	0.045	0.0030	0.04750.0060
S7(40-0.01)	0.042	0.0026	0.042+0.0052	0.030	0.0026	0.30+0.0052

Table 14: Estimated values of standard deviation.

B. Interaction Cadmium/Copper tests:

The second element selected for our investigation is Copper, because this element is also a desirable element for biological systems on the one hand and presents probable competitions with toxic elements in biological media on the other hand; thus, monitoring the protocol will help us in our approach to derive the best empirical results based on the comparison of the behaviors of our contamination bioindicator.

B.2.1 Test 1: Interaction Cadmium/ Copper: Concentration Cadmium: 0.01 ppm Concentrations Copper: 1-5-10-15-25-30-40 ppm

Samples [Cu]/[Cd] ppm	D1	D2	D3	D4	D5	D6	D7
W.pt	-	-	-	-	-	-	-
S1 (1-0.01)	-	-	-	SL	SL	SL	SL
S2 (5-0.01)	-	SL	SL	SL+Chl	SL+Chl	SL+Chl	SL+Chl
S3 (10-0.01)	BC	SL	SL	SL+Chl	SL+Chl	SL+Chl	SL+Chl
S4 (15-0.01)	BC	SL	SL	SL+Chl	SL+Chl	SL+Chl	SL+Chl
S5 (25-0.01)	BN+BC	BN+BC	BN+BC	BN+BC	BN+BC	SL+Chl	Chl
S6 (30-0.01)	BN+BC	BN+BC	BN+BC	BN+BC	BN+BC	BN+BC	Chl+DN
S7 (40-0.01)	BN+BC	BN+BC	BN+BC	BN+BC	BN+BC	BN	BN

Table 15: Visual observations Test 1

Samples [Cu]/[Cd] ppm	S. Pt (Cm) B. F	S. Pt (Cm) A. F	F.W Pt (g) B.t	F.W Pt (g) A.t	D.W Pt(g)	NS. V (mi) A.t
W.pt	13.50	14.50	1.70	2.25	0.15	75
S1 (1-0.01)	13.5	14.20	1.80	2.17	0.12	70
S2 (5-0.01)	14.25	14.80	1.54	1.75	0.14	72
S3 (10-0.01)	12.80	13.20	1.90	2.45	0.15	78
S4 (15-0.01)	13.40	14.19	1.74	1.85	0.14	72
S5 (25-0.01)	12.20	13.10	1.54	1.95	0.18	79
S6 (30-0.01)	14.12	15.50	2.11	2.62	0.19	80
S7 (40-0.01)	13.50	13.50	1.81	2.38	0.10	90

Table 16: sizes of plants before and after transfer with fresh and dry weight of plants and volume of nutrient solution Test 1

Samples [Cu]/[Cd] ppm	Abs (pt)	Abs (SN)	[Cd] Pt ppm	[Cd] SN ppm	% Recuperation
W.pt	0.01	0.32	00.0	0	-
S1 (1-0.01)	0.06	0,98	0.98	0.64	89
S2 (5-0.01)	0.01	0,91	0.91	0.52	87
S3 (10-0.01)	0.02	0,99	0.99	0.45	94
S4 (15-0.01)	0.04	1,03	1.03	0.38	76
S5 (25-0.01)	0.05	1,09	1.09	0.26	91
S6 (30-0.01)	0.06	1,11	1.11	0.16	89
S7 (40-0.01)	0.07	1,20	1.2	0.1	87

Table 17: Concentrations determined by AAS Test 1

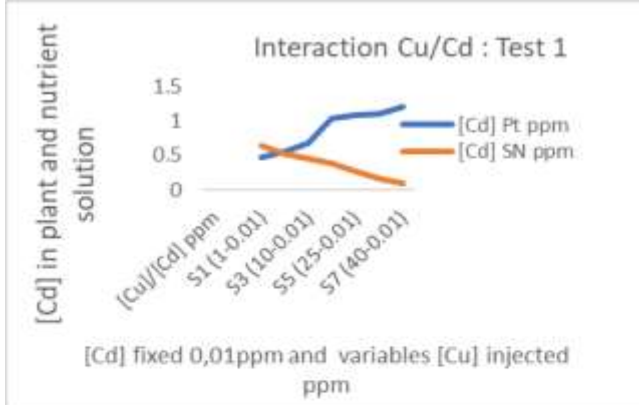

Fig 5: Interaction Cd/Cu Test 1

	Plante			Nutrient Solution		
Samples [Zn]/[Pb] ppm	X	SD	X+-2SD	X	SD	X+2SD
W.pt	-	-		-	-	-
S1 (1-0.01)	0.048	0.0028	0.048+0.0116	0.068	0.0052	0.068+0.0104
S2 (5-0.01)	0.060	0.0039	0.060+0.0050	0.062	0.0051	0.062+0.0102
S3 (10-0.01)	0.061	0.0025	0.061+0.0050	0.055	0.0049	0.055+0.0
S4 (15-0.01)	0.067	0.0048	0.067+0.0096	0.069	0.0028	0.069+0.0056
S5 (25-0.01)	0.072	0.0052	0.072+0.0076	0.047	0.0046	0.047+0.0092
S6 (30-0.01)	0.075	0.0049	0.07540.0062	0.041	0.0031	0.041+0060
S7 (40-0.01)	0.079	0.0055	0.079+0.0052	0.028	0.0027	0.028+0.0052

Table 18: Estimated values of standard deviation Test 1

B.2.2 Test 2: Interaction Cadmium/ Copper: Concentration Cadmium: 0.01 ppm Concentrations Copper: 1-5-10-15-25-30-40 ppm

Samples [Cu]/[Pb] ppm	D1	D2	D3	D4	D5	D6	D7
W.pt	-	-	-	-	-	-	-
S1 (1-0.01)	BC	BC	SL+Chl	SL	SL	SL	SL
S2 (5-0.01)	BC	BC	SL	SL+Chl	SL+Chl	SL+Chl	SL+Chl
S3 (10-0.01)	BC	BC	SL	SL+Chl	SL+Chl	SL+Chl	SL+Chl
S4 (15-0.01)	BC	BC	SL	SL+Chl	SL+Chl	SL+Chl	SL+Chl
S5 (25-0.01)	BC+BN	BC+BN	BC+BN	BC+BN	BC+BN	SL+Chl	Chl
S6 (30-0.01)	BC+BN	BC+BN	BC+BN	BC+BN	BC+BN	BC+BN	Chl+BC
S7 (40-0.01)	BC+BN	BC+BN	BC+BN	BC+BN	BC+BN	N	N

Table 19: Visual observations Test 2

Samples [Cu]/[Cd] ppm	S. Pt (Cm) B. F	S. Pt (Cm) A. F	F.W Pt (g) B.t	F.W Pt (g) A.t	D.W Pt(g)	NS. V (mi) A.t
W.pt	12.50	1.50	1.55	1.95	0.12	73
S1 (1-0.01)	11.5	12.20	1.60	1.87	0.14	71
S2 (5-0.01)	13.75	14.20	1.65	1.42	0.16	74

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S3 (10-0.01)	13.50	14.20	2.40	2.90	0.13	76
S4 (15-0.01)	13.10	13,90	1.63	1.96	0.15	77
S5 (25-0.01)	12.85	13.46	1.76	12,15	0.16	80
S6 (30-0.01)	13.87	14.62	1.96	2.23	0.17	82
S7 (40-0.01)	12.98	13.75	1.95	2.16	0.10	87

Table 20: sizes of plants before and after transfer with fresh and dry weight of plants and volume of nutrient solution Test 2

Samples [Cu]/[Cd] ppm	Abs (pt)	Abs (SN)	[Cd] Pt ppm	[Cd] SN ppm	% Recovery
W.pt	0.01	0.28	00.0	0	-
S1 (1-0.01)	0.08	0,78	0.86	0.56	91
S2 (5-0.01)	0.03	0,83	0.91	0.48	92
S3 (10-0.01)	0.04	0,84	0.85	0.44	90
S4 (15-0.01)	0.07	0.95	0.93	0.31	84
S5 (25-0.01)	0.09	0.96	0.97	0.21	85
S6 (30-0.01)	0.09	0.98	0.99	0.20	86
S7 (40-0.01)	0.10	0.98	1.10	0.16	90

Table 21: Concentrations determined by AAS Test 2

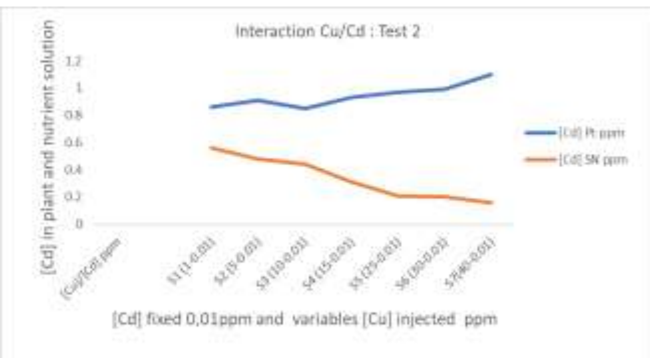

Fig. 6: Interaction Cd/Cu Test 2

Plante				Nutrient Solution		
Samples [Zn]/[Pb] ppm	X	SD	X+2SD	X	SD	X+2SD
W.pt	-	-		-	-	-
S1 (1-0.01)	0.046	0.0022	0.046+ 0.0116	0.072	0.0048	0.072+ 0.00115
S2 (5-0.01)	0.049	0.0045	0.049+ 0.0050	0.063	0.0040	0.063+ 0.072,
S3 (10-0.01)	0.059	0.0034	0.059+ 0.0050	0.052	0.0056	0.052+ 0.0070
S4 (15-0.01)	0.064	0.0028	0.064+ 0.0096	0.071	0.024	0.071+ 0.0048-
S5 (25-0.01)	0.072	0.0041	0.072+ 0.0076	0.043	0.0048	0.043+ 0.0092
S6 (30-0.01)	0.077	0.0054	0.072+ 0.0062	0.038	0.0036	0.038+. 0060
S7(40-0.01)	0.081	0.0058	0.081+ 0.0052	0.031	0.0032	0.031+ 0.0052

Table 22: Estimated values of standard deviation Test 2

C. Discussion of results:

According to the results obtained from tests carried out on the plant Phaseolus–vulgaris, we note that zinc gives satisfactory results in terms of reduction of the harmful action of Cadmium, this amounts to bringing these physico-chemical properties closer together [4] and [20]. With those of Cadmium on the one hand and it's desirable action of living organisms on the other [9]. One can notice from the aberration of tables 7 Test 1 et Table 11 Test 2, that the symptoms of toxicity of the Phaseolus-Vulgaris plants exposed to the toxic Cadmium in the presence of Zinc at different concentrations, were delayed in appearing by the plants contribution to the plants exposed to the Cadmium injected while alone.

This delay can be explained by the effect of Zinc on the absorption of Cadmium by preventing it from being absorbed by the plant, in other words Zinc exerts an antagonistic effect vis-à-vis the availability of Cadmium , the visual observations are consolidated by the empirical results, that is to say the determination of the quantity of Cadmium absorbed by the plant and that retained in the nutritive solution, by means of atomic absorption spectroscopy, which confirms to us according to the respective readings of each sample, the antagonistic effect of Zinc in competition with the toxic Cadmium see tables 8-9 Test 1 and 12-13 Test 2 ; Fig 3 Test 1 and Fig 4 Test 2, results consolited by Estimated values of standard deviation Table 10 Test 1 and Table 14 Test 2 , result which agrees with the work of [18] Moreno- Jimenez. E and al ; 2016 ; who confirmed that Zinc is highly phyto-available, and is found in higher amounts in the roots than in the aerial parts in addition a recent study by [22] mentioned that soluble forms of Zn are readily available to plants. Some authors have spoken of the antagonistic action of Zn in relation to the absorption of Cd and consequently have demonstrated a protective role of Zn in plants exposed to oxidative stress induced by Cd. [13],[5].

The modifications recorded during the investigation are therefore:

• Decrease in the assimilation of Cadmium
• Delay in the onset of symptoms of toxicity, which can be explained by:

- The inhibitory action of Zinc vis-à-vis the absorption of Cadmium.
- Stimulation of the enzymatic activity of the plant by the contribution of Zinc, and the safeguard of the chlorophyllin apparatus against the chlorotic action of Cadmium on the plant [33].

This is how we can conclude that an interaction already occurs between the two elements Cadmium / Zinc, since the amount of Cadmium retained in the nutrient solution is much higher than that absorbed by the plant, which can be explained by the pre-treatment of Zinc which is carried out during all the interaction experiments between Cadmium and Zinc. This pretreatment of Zinc injected 24 hours before the injection of the toxic Cadmium, this pretreatment was a positive contribution; since it led on the one hand to provide resistance to the plant [8]], and by inducing the formation of metallothionine, this enzyme which is at the origin of the tolerance of the plant towards the toxic [28] ,The Cd/Zn complex, is able to accumulate significant amounts of these elements without exhibiting growth inhibition or increased mortality [21]. Zn protects chloroplast photochemical functions from Cd toxicity: Metal ions are well known to affect the structure and function of chloroplasts in many plant systems such as Beta vulgaris [31].

Zinc prefers binding to the –SH groups of the membrane protein moiety and protects the phospholipids and proteins from thiol oxidation and desulphated formation [11].

Cd is known to affect enzymes and proteins through its interaction with –SH groups as well as inducing redox cycling [25]. Moreover, the S-P orbital energy separation for Cd is less than that of Zn, suggesting that excitation of the valence state may be easier for Cd than for Zn due to its low charge density [37] ; Zinc prefers binding to the –SH groups of the membrane protein moiety and protects the phospholipids and proteins from thiol oxidation and desulphated formation [23] .

Cd is known to affect enzymes and proteins through its interaction with –SH groups as well as inducing redox cycling [35]. Moreover, the S-P orbital energy separation for Cd is less than that of Zn, suggesting that excitation of the valence state may be easier for Cd than for Zn due to its low charge density.

On the other hand, the results of the Cadmium / Copper interaction through the investigations on the plant Phaseolus-vulgaris, show that the effects are synergistic and this appears according to the tables 15 - 17 and figures 5 test 1 and tables 19-21 figure 6 test 2

This synergistic action is due to the properties of copper in competition with cadmium in the same biological organism case of the investigative plant Phaseolus-vulgaris, the potentiation of the synergistic effect is illustrated by tables 15 (visual observations) -17 (Concentrations determined by AAS) and figure 5 for test 1 and tables 20 (visual observations) -21 (Concentrations determined by AAS) and figure 6 for test 2.

The gradual potentiation of the effect is probably due to the fact that Copper is responsible for an inhibition of photosynthesis by the peroxidation of lipids in thylakoid membranes, blocking the activity of ribulose 1-5-biphosphate carboxylase–oxygenase [14] and [30].

It would be probable that the effects of Cd and Cu are the consequence of an inhibitory action, direct and / or indirect on the proteolysis.**[**6] have also shown that Cd and Cu can disrupt protein metabolism during lentil seed germination. [32] and [33] demonstrated that the toxicity of Cu^{2+} induces a reduction in photosynthetic activity by a decrease in the quantum yield of the photosystem.

We can also note through our investigation, that copper exhibits harmful actions from a concentration of 15 ppm; which is exactly in line with the work of [3] A indicate that the threshold of Cu toxicity is reached from a Cu concentration of 15 to 30 mg / kg DM in the leaves of cultivated plants. And the work of [7]and [36] who’s concluded that the Cu content observed in the plant can reach a critical concentration, which varies according to the plant species, from which symptoms of phytotoxicity appear [16].

It would be probable that the effects of Cd and Cu are the consequence of an inhibitory action, direct and / or indirect on the proteolysis [27] have also shown that Cd and Cu can disrupt protein metabolism during lentil seed germination.

The reliability of the empirical results is verified by the results of the estimated values of standard deviation.

In this context of study carried out in vitro informed us by this original approach that the result obtained by this investigation in terms of interaction is of great importance in order to manage cadmic contaminations.

At the end, we can point out that the originality of our investigation lies in the rational use of our own means (culture chamber) and the permanent monitoring of the batches of investigations with permanent verification of intrinsic and extrinsic factors to avoid probable interference.

IV. CONCLUSION

After having fixed the minimum concentration of Cadmium which induce the first symptoms of toxicity vis-à-vis the plant Phaseolus-Vulgatis which is around 0.01 ppm.

From this study it emerges that Cadmium is a very potent toxic to all biological systems, since its minimum concentration can cause symptoms of toxicity towards the plant Phaseolus Vulgaris is around 0.01 ppm.

The content of 0.01 ppm is explained by the fact that the presence of cadmium in the germination medium significantly limits the rehydration of the seed coats, and causes reduction of the germination rate and root growth. In plants, cadmium has no known biological function [34].[9] Cadmium also causes a reduction in transpiration as well as an increase in stomatal resistance accompanied by an increase in the content of abscisic acid. (ABA) [18]. Thus, by studying its interaction with a second divalent metal which is Zinc in the same biological system, we could deduce the positive effect of the latter by acting as an inhibitor on the transport of Cadmium.

The study of the interaction between Cadmium and Zinc, the latter which was injected at different pre-established concentrations and varying from 1 to 40 ppm, was of positive benefit for the plant by acting as a competitor with Cadmium in preventing it from being absorbed in large quantities, this is illustrated by visual observations, as well as empirical results (SAA), it is at this level that we could confirm the effect of Zinc on the retention of Cadmium in the plant.

This hypothesis agrees perfectly with the fact that certain compounds, including thiols and zinc, have an antagonistic effect with respect to the toxicity of cadmium.

Thus, the convergence of our results with those of several researchers, namely [17] which by studying this type of interaction leads to the same conclusions.

Therefore, the contribution of Zinc during Cadmic poisoning is of great importance, since Zinc plays a preventive role in this kind of poisoning in the long term.

Therefore, the Cadmium / Zinc relationship or interaction is an important factor in this regard.

Regarding the Cadmium / Copper interaction, the results obtained by the investigation tests on the Phaseolus-vulgaris plant have shown us the opposite effect: that copper potentiates the synergistic effect of cadmium.

We therefore retain through our investigation focused on cadmium inhibition tests by a selective Cadmium / Zinc and Cadmium / Copper investigation in the plant selected for the investigation that Zinc minimizes the toxic action of Cadmium while Copper potentiates this same effect, results confirmed by visual observations and consolidated by the empirical results with verification of the degrees of error in two iterative tests.

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Author Contributions:

1. Dr. Sahraoui Nabil: Responsible for the research protocol in the field of in-vitro plant culture, daily monitoring with note taking of the state of the plants, in addition to doing the analysis of samples by atomic absorption spectroscopy, and interpretation of the results.

2. Dr. Abdeddaim Mohammed: Preparation of the plant samples for analysis, in addition to doing the calibration of the instrument and assistance in the area of interpretation of the results.

3. Dr. laidoune Abdelbaki: Data verification, calculation of estimated values of the standard deviation, as well as assistance in the formatting of the article.