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Impact of CdCl₂ on hematological and biochemical parameters of rabbits *Oryctolagus cuniculus* and opposite effect of Ca²⁺

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ABSTRACT

Heavy metals and its derivatives are a special class of toxics. Many metals are useful for industrial, agricultural and medical applications. Indeed, they can go back through the food chain and thus achieve to human being. Our aim is to study an example of heavy metals "Cadmium" CdCl₂ at two concentrations 30 and 60 ppm on biological model *Oryctolagus cuniculus* that is very used in Toxicological studies, and the possible neutralization using the Ca²⁺.

Our results showed a significant increase in the weight of liver, this augmentation is corrected with the addition of Ca²⁺ for the lowest concentration 30ppm and in the 07th day, but over that there is no effect of calcium on the toxicity of Cd, the biochemical and hematological parameters generally are disturbed and the effect of calcium is less or more shown in the most experiments. © 2013 Trade Science Inc. - INDIA

KEYWORDS

Oryctolagus cuniculus;
Heavy metals;
Cadmium;
Calcium;
Hematology;
Protein;
Detoxification.

INTRODUCTION

In our environment, we are exposed to a number of natural or synthetic substances that can be caused toxic effects, and heavy metals are of these harmful substances^[2].

Heavy metals known as natural metallic elements having a density exceeded 5g/cm³. These are most often present in the environment astraces, mercury, lead, cadmium, copper, arsenic, nickel, zinc, cobalt, manganese are example. Most of them are toxic like lead, cadmium and mercury^[32]. We are interested in our work

to cadmium; it has many similarities with the physical and chemical character as zinc and is found in nature accompanying zinc.

Cadmium is highly corrosion, resistant and has been widely used in electroplating of other metals, mainly steel and iron. However, currently, only 8% of the total refined cadmium are used for veneers and coatings. Cadmium compounds (30% of its applications in developed countries) are used as pigments and stabilizers in plastics. Cadmium is also used in some alloys (3%). Small cadmium batteries, rechargeable and used, for example, in mobile phones, contribute to the rapid increase in the

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use of cadmium (in 1994, 55% of cadmium in industrial countries were in the batteries)^[11].

The toxicity of cadmium varied and depended to the chemical state and the duration and amount of exposure. It causes tubular dysfunction that result in increased secretion of low molecular weight proteins in the urine. Cadmium may also cause disturbances of calcium metabolism. High exposure to cadmium, most probably related to other factors such as dietary deficiency can lead to osteoporosis and/or osteomalacia. The inhibitory effects of cadmium on reproductive cells are proven in rodents and suspected in humans^[11].

Rabbits have been domesticated since the sixth century, when they were kept for food and fur. They have been also selectively bred over the years for varieties in the fur and are a popular pet.

After mice and rats, they are the most common laboratory animal. As many as 76 different breeds of rabbit are known by the British Rabbit Council intended, the New Zealand White (NZW), bred in the 1920s has become the one most commonly used in research.

Historically, they have been most used for antibody development and testing as sentinels for a wide array of products^[27]. The rabbit (*Oryctolagus cuniculus*) is widely used as a model for human disease because of its size, physiological attributes, and similar disease characteristics^[19].

In immunology, Gertz et al.^[8] worked on the regions encoding the coordinately regulated Th2 cytokines IL5, IL4 and IL13 of the rabbit *Oryctolagus cuniculus* by comparing sequences of syntenic regions on chromosome 3, and they identified several differences between the two donor rabbits in coding and non-coding regions of potential functional significance, confirmation awaits additional sequencing of other rabbits.

Rabbits can be restrained in stocks and easily generally docile and are cheap to maintain, they have been used for a wide-range of toxicity testing, especially on their skin^[26].

Anjum^[7] found that rabbits are excellent models for investigation of heavy metals effects on liver functions and drug metabolism enzyme system, where he found that the addition of CdCl₂ to the rabbits pretreated with phenobarbitone and promethazine with dose of 5mg/kg of weight increases the activities of serum GOT, LDH and ICDH 49%, 73% and 32%, respectively.

Activity of AP was decreased 69% in the phenobarbitone. In pretreated promethazine rabbits, cadmium chloride administration decreased the activities of serum GOT, GPT, LDH and AP, 56%, 35%, 27% and 25%.

Eira et al.^[6] searched the concentration of some toxic elements in *Oryctolagus cuniculus* and in its intestinal cestode *Mosgovoyia ctenoides*, in Dunas de Mira (Portugal), The highest quantity of Pb was found in rabbit muscle (3.81 ppm) while highest Cd and Hg values were found in kidney (1.02 and 0.08 ppm).

In our work we have tried to highlight the effect of cadmium at two concentrations 30 and 60 ppm on rabbits (*Oryctolagus cuniculus*) and the possible role of calcium in the phenomenon of detoxification.

MATERIALS AND METHODS

Biological material

For our experiments, we chose to work on rabbits of local strain in the region of Tebessa east-north Algeria. All rabbits were males weighing between 260 g and 760 g and have a soft fur reddish brown, black, white and gray. They had access to water and their food for all times. Animals were kept under constant conditions of temperature environ 25±3°C and humidity 35±5%. The total body weight of body was daily recorded before and during the experiments. There was a gain in body weight and increase of food consumption indicating the good conditions of laboratory.

Chemical

We used a cadmium under Cadmium Chloride form. Aqueous solutions of cadmium chloride salt was administered by oral system. Control rabbits were kept untreated and their body weight was recorded daily, we selected two doses 30 and 60ppm.

METHOD

Description and treatment

To begin our experience, we have handled 63 rabbits of local breed in the region of Tebessa (*Oryctolagus cuniculus*). These rabbits were divided on 6 lots of 9 rabbits and we kept nine rabbits as control. The treatments began 15 days (adaptation period of rabbits) as

follows.

- Lot1 : as controls without treatment
 Lot2 : treated of 30 ppm CdCl₂
 Lot 3 : treated of 60 ppm CdCl₂
 Lot4 : treated of 30 ppm Ca²⁺
 Lot 5 : treated of 60 ppm Ca²⁺
 Lot6 : treated of CdCl₂ / Ca²⁺ 30 ppm
 Lot7 : treated of CdCl₂ / Ca²⁺ 60 ppm

All the animals were killed by cervical dislocation 24 hours after last treatments. The blood samples were for estimation of hematological parameters.

Liver was taken out for biochemical tests, relative weight of liver was estimated by the following formula: RLW=(liver weight/body weight) X100. Liver protein level was measured by Bradford (1976) method.

Hematological study method

The hematological parameters hemoglobin, MetHb and a parameter considered as an early marker of inflammation (erythrocyte sedimentation level). Blood sampling was done at the laboratory of the University of Tébessa. The first sampling on 7thday, the second to the 16thday and the last 21stday of treatment.

Determination of methemoglobin (MetHb)

The procedure consists of two steps

First step

0.2 ml of heparinized blood and 5 ml of isotonic chloride (9%), Centrifugation at 5000 rpm for 5 min-

utes, Base with 5 ml of ice water for 15 minutes, Complete with 10 ml of Sorensen's buffer and agitation followed by centrifugation 5000 rpm for 5 minutes then read the optical density of the supernatant at 632 nm (ODA).

Add a drop of the mixture (KCN [0.1 M] + CH₃COOH [12%]) to the supernatant and read the optical density (ODB)

Second step

Add to 2ml of supernatant 1.5 ml of Fe (CN) 6 K3 (brown color), and read the optical density (ODC).

Add a drop of mixture (KCN [0.1 M] + Fe (CN) 6 K3) (red color), and read the optical density (ODD).

The level of methemoglobin is obtained by the following formula:

$$\text{MetHb} = [(ODA-ODB) / (OD C-ODD)] \times 100$$

RESULTS

Effects of CdCl₂, on hemoglobin (Hb) and the role of Ca²⁺

TABLE 1 shows the effects of CdCl₂, and the role of Ca²⁺ in the 7th, 16thand 21stdays of treatment. There is a decrease of hemoglobin levels in all treated animals with CdCl₂ (P=0.001). The addition of Ca²⁺ increases this level as controls level. The Dunnett's test confirms that there is a difference compared to controls of treated by CdCl₂.

TABLE 1 : Effect of CdCl₂ on hemoglobin Hb and the role of Ca²⁺ (g/dl).

Sample (g/dl)	Control	CdCl ₂ 30ppm	CdCl ₂ 60ppm	Ca ²⁺ 30ppm	Ca ²⁺ 60ppm	CdCl ₂ /Ca ²⁺ 30ppm	CdCl ₂ /Ca ²⁺ 60ppm
7 th day	11.600±0.530	8.000±0.500*	7.600±0.530*	11.00±0.500*	11.66±0.290	11.530±0.500	11.400±0.530
16 th day	11.530±0.500	9.200±0.260**	7.260±0.250**	12.530±0.550*	11.850±0.300*	10.460±0.0.500*	12.570±0.560*
21 st day	11.430±0.513	8.800±0.755*	8.400±0.535*	11.27±0.374	12.50±0.500	11.610±0.548*	11.140±0.17

* (p<0.05) There is a significant difference between control and treated sample; ** (p<0.01) there is very significant difference between control and treated samples.

Effects of CdCl₂, on Sedimentation speed (SV) level and the role of Ca²⁺

First hour

The effects of chloride cadmium on 1st hour sedimentation speed level of red blood cells and the role of calcium in the detoxification is showed in the TABLE 2. In the 7thday there was an increase of this

parameter in organisms treated by(30 and 60 ppm)CdCl₂, Ca²⁺ (30 ppm) and combined treatment (30, 60 ppm), in the rest of parameters there is a decrease (P = 0.301). at the 16th day there was an increase in the two CdCl₂ concentrations, Anova test shows that there is no difference in the 7thand 16thday compared to the controls, except the 21st where there a difference.

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TABLE 2 : Effect of CdCl₂ on the sedimentation speed level of the RBC at 1st hour (mm/h).

Samples (mm/h)	Control	CdCl ₂ 30ppm	CdCl ₂ 60ppm	Ca ²⁺ 30ppm	Ca ²⁺ 60ppm	CdCl ₂ /Ca ²⁺ 30 ppm	CdCl ₂ /Ca ²⁺ 60 ppm
7 th day	2.000±1.000	3.330±1.582	4.330±2.517	4.330±2.528	1.660±1.155	3.330±1.528	3.000±1.000
16 th day	2.000±1.000	3.000±1.000±	2.330±1.155	1.330±1.528	1.330±1.528	1.330±0.577±	3.660±1.155
21 st day	2.000±1.000	2.000±1.000	3.000±2.000	5.000±1.000**	1.660±1.155	1.330±0.577*	1.000±0.000*

**($p < 0.01$) there is a high significant between control and treated samples.

Second hour

The effects of CdCl₂ on the level of sedimentation at the second hour are shown in (TABLE 3). In general manner, there is a big increase of the sedimentation speed level in all cadmium treatments, but the role of calcium is very clear where there is a neutralization effect. Dunnett's test confirms that there is a difference compared to the control, except to all treated with Ca²⁺ ($p > 0.05$).

TABLE 3 : Effect of CdCl₂ on the sedimentation speed rate of RBC in the second hour (mm/h).

Samples (mm/h)	Control	CdCl ₂ 30 ppm	CdCl ₂ 60 ppm	Ca ²⁺ 30 ppm	Ca ²⁺ 60 ppm	CdCl ₂ /Ca ²⁺ 30 ppm	CdCl ₂ /Ca ²⁺ 60 ppm
7 th day	2.330±0.577	5.330±2.082*	7.000±1.000**	2.360±1.155	2.000±0.000	3.660±1.528	3.640±1.528
16 th day	2.340±2.517	4.990±1.155*	6.000±1.000*	1.660±2.082	1.660±1.528	2.250±1.000	3.660±1.528
21 st day	2.330±0.577	3.240±1.000	4.660±1.528*	2.690±1.155	2.660±1.155	2.330±0.577	1.660±0.577

organisms treated by cadmium are differences to the control.

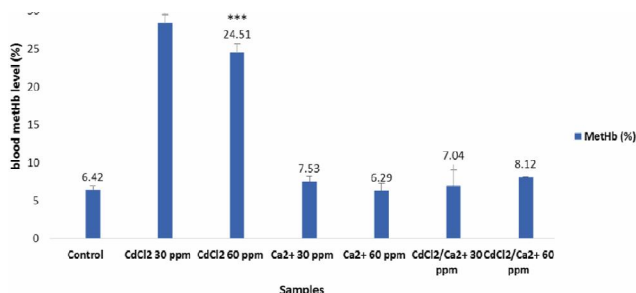


Figure 1 : Effect of CdCl₂ on methemoglobin level and the role of Ca²⁺.

Effects of CdCl₂ on relative liver weight and the role of Ca²⁺

Impact of chloride cadmium on relative liver weight

TABLE 4 : Effects of CdCl₂ on relative liver weight (g).

Samples x100 (g)	Control	CdCl ₂ 30ppm	CdCl ₂ 60 ppm	Ca ²⁺ 30 ppm	Ca ²⁺ 60 ppm	CdCl ₂ /Ca ²⁺ 30 ppm	CdCl ₂ /Ca ²⁺ 60 ppm
7 th day	0.020±0.003	0.035±0.002**	0.040±0.002***	0.022±0.003	0.020±0.002	0.020±0.004	0.024±0.003
16 th day	0.020±0.002	0.032±0.001**	0.044±0.001***	0.026±0.001	0.028±0.001	0.023±0.001	0.025±0.002
21 st day	0.025±0.005	0.039±0.004**	0.049±0.005***	0.029±0.004	0.030±0.003	0.026±0.002	0.027±0.004

***($P < 0.001$) very high significant difference

Effects of CdCl₂ on methemoglobin level and the possible role of Calcium

The results shown in (figure 1) illustrated the effects of chloride calcium on the level of methemoglobin and the role of calcium in the correction of effects. There was an increase in all CdCl₂ treated versus control ($p < 0.001$). The role of Ca²⁺ was very clear in the correction of this intoxication. Dunnett's test confirms that

is shown in the TABLE 4. All treatments by CdCl₂ showed an augmentation in RLW, the Ca²⁺ decrease and correct this amount according to the treatments ($p > 0.05$).

Effect of CdCl₂ on hepatic protein level and the role of Ca²⁺

The protein content of rabbit liver was determined using Bradford method, and the effects of CdCl₂ and the possible opposite effect of Ca²⁺ are given in the figure 2. We observe a very high significant increase ($p < 0.001$) of protein level in all treaties by CdCl₂. This increase is correct significantly by the addition of Ca²⁺. The Dunnett's test and analysis of variance shows that there is a significant difference between Cd treaties and control.

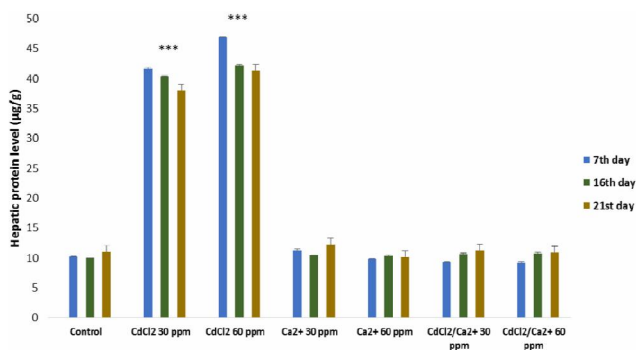


Figure 2 : Effects of 30 and 60 ppm of CdCl₂ on hepatic protein level (µg/g) and the role of Ca²⁺.

DISCUSSION

This study aimed to highlight a possible toxicity of cadmium CdCl₂ on some hematological and biochemical parameters of *Oryctolagus cuniculus* as a biological model, and the possible role of Calcium Ca²⁺ in the detoxification and neutralization of these effects.

Cadmium is reported having no known beneficial functions in animal life^[31]. Once in the system cadmium binds with enzymes having sulfhydryl groups^[13], disturbs cell membrane permeability^[15], deposits in cellular organist and binds with nucleic acids^[25]. All the biological functions like excretion, digestion, respiration and reproduction are affected by the intoxication by cadmium causing the death of organism.

Blood is one of the most sensitive indicators of many metabolic disorders^[17,29]. Numerous studies have shown that the primary site of toxic action in the body is the red blood cells and hemoglobin which more precisely the role of carrying oxygen when the iron is under ferrous form Fe²⁺^[18,20].

Cadmium oxidizes ferrous iron Fe²⁺ active molecule of hemoglobin to ferric iron Fe³⁺ inactive and the resulting molecule is called methemoglobin which is incapable of reversibly binding oxygen^[5,14]. Our results showed a toxic effect of cadmium translated by the increase in methemoglobin (MetHb); these effects are corrected by the adding of the Calcium Ca²⁺. In adults, there are enzyme systems such as NADH-MetHb reductase; formed methemoglobin eliminating the absence of this enzymatic equipment in the fetus resulting in cyanosis and high neonatal mortality. Ampy and Williams^[1] showed that nitrates generate a cascade of

physiological phenomena affecting in most cases the blood tissue but also other organs (kidney, spleen and liver).

The addition of Ca²⁺ reduced the effect of CdCl₂, corrected the level of MetHb, probably by conserving the enzymatic compartment, and enhanced the eliminating of CdCl₂ by exocytosis phenomenon.

Our results showed a decrease of hemoglobin level in treated animals by cadmium, Sutton^[28] found a decrease in hemoglobin and hematocrit in female mice that are exposed to a dose of 6 mg/kg of Cd. Prigge^[21] exposed female rats at concentrations Cd from 25 to 50 mg/m³ (as Cd oxide) for 100 days also cause a reduction in growth and an increase in hemoglobin and hematocrit.

The addition of Ca²⁺ to the cadmium's treated animals returns the amount of Hb to normal level and corrected the effect of this metal. Several hypotheses, sometimes contradictory, have been proposed to explain the inhibitory action of calcium on the collection and accumulation of cadmium. Knowing that Cd²⁺ ions can be transported through the protein calcium channels in the membranes of root cells^[30] a possible competition between the two ions can occur for the same absorption sites^[34,16]. Calcium is a competitive inhibitor of cadmium uptake in *Rhytidadelphus squarrosus*^[33].

In present work when the body of animals were dissected, in most cases, their abdominal cavities were filled with fluids, liver were shrunk, having numerous white spots probably fats infiltrations. At sublethal doses there was no changes in body weights suggesting that the doses was not strong enough to produce the known cadmium symptoms as skeleton deformation and renal disorders^[22]. However, metal after ingestion induces the increase of relative liver weight this results are in concordance with the results of Grose et al.^[13]. Goering and Curtin^[10] reported moderate to severe hepatic injury, evident by cells swelling after cadmium administration to immature rats. Cadmium was reported to inhibit protein synthesis at cellular level^[35] which probably returns on hepatic weight.

The liver shows a change in weight after ingestion of cadmium. Borzelleca et al.^[4] found effects on growth and organ weights of spargue-Dawley rats who ingested doses of Cd about 1.1 to 14 mg/kg for 10 days in their drinking water, and from 15 to 65 mg/kg, organ weights

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were also modify. The presence of Ca²⁺ combined with CdCl₂ corrected the decrease of liver weight to normal level, this due probably by the competition and the role of calcium in the cadmium extracellular movement, and the sedimentation speed confirm that there was a hepatic injury; this is corrected by the calcium combination.

The rate of hepatic protein was increased in the animals treated with CdCl₂, this augmentation is the results of the resistance enzymes secretion^[24]. The cadmium perturbs all metabolic ways in the organism, and the Ca²⁺ correct these perturbation.

The presence of calcium associated with doses of cadmium in our experiments induced a correction of cadmium effects at 30 and 60ppm. These results are consistent with the results of Raghpathy and Nasa^[23] were exposed rats to 25 ppm of CdCl₂ with drinking water hanging 8 weeks, groups fed with low calcium diet (0.1 %) had increased retention of cadmium and cadmium toxicity compared to groups that were fed with diet of high calcium (0.6%). We can say that the calcium maintains low concentrations of metal in the cytosol. These results are consistent with the work of Zoghlami et al.^[36] have shown that cadmium causes an inhibition of weight gain that depends on the concentration of the metal in the organ.

As conclusion, many negative effects of cadmium can be corrected and neutralized with the best amount of calcium.

REFERENCES

- [1] F.R.Ampy, A.D.Williams; Dimerhyll nitrosamine metabolism. *In vitro* activation of dimethylnitrosamine to mutagenic substance by hepatic and renal tissue from 3 inbred of mice. Ed. Life Sciences, 923-930 (1986).
- [2] D.F.Basketter, I.Gerberick, C.Willis; Toxicology of contact dermatitis: Allergy and urticaria. Ed. Current toxicology series, 180 (1999).
- [3] C.Briefert et, R.Perraud; Chimie de l'environnement Air-Eau-Sols, sols -déchets. Ed. ADEME, 369-387 (2003).
- [4] J.F.Borzelleca, J.W.Olson, F.E.Reno; Lifetime toxicity/carcinogenicity study of FD & C red No. 40 (allura red) in Sprague-Dawley rats. Food Chem. Ed. Toxicol, 27, 701-705 (1989).
- [5] M.A.Doland, N.C.Luban; Méthemoglobinemia in two children: Disparate etiology and treatment, (Eds); *PediatEmerg*, 171-175 (1987).
- [6] C.Eira, J.Torres, J.Vingada, J.Miquel; Concentration of some toxic elements in *Oryctolagus cuniculus* and in its intestinal cestode *Mosgovoyia tenoides*, in Dunas de Mira (Portugal). *Sci Total Environ*, 346(1-3), 81-6, Jun 15 (2005).
- [7] Farah.Anjum; EFFECT OF HEAVY METAL TOXICANTS ON LIVER FUNCTION AND HEPATIC DRUG METABOLIZING ENZYME SYSTEM IN RABBIT Institute/University/Department Details Department of Zoology University of the Punjab, Lahore Session 1991 Subject Zoology, 370 (1991).
- [8] E.Gertz, AgarwalaRicha.Michael, G.Mage.Rose, A.Schäffer.Alejandro; Comparative Analysis of Genome sequences of the Th2 cytokine Region of Rabbit (*Oryctolagus cuniculus*) with those of nine Different species. *Immunology and Immunogenetics Insights*, 3, 59-82 (2011).
- [9] G.A.Giessler, N.M.Gades, P.F.Friedrich, A.T.Bishop; Severe tacrolimus toxicity in rabbits. Source Department of Orthopedic Surgery, Microvascular Research Laboratory, Mayo Clinic, Rochester, MN 55905, USA.
- [10] P.L.Goering, D.L.Curtin; Resistance of cadmium induced hepatotoxicity in immature rats. *Toxicol.Appl.Pharmacol*, 74, 321-329 (1984).
- [11] C.Grant, L.D.Bailey, F.Selles; Cadmium accumulation in corps. Ed. Work-Shop Proceedings, 55-71 (1995).
- [12] C.Grant, L.D.Bailey et, F.Selles; Cadmium accumulation in corps. Ed. Work-Shop Proceedings, 55-71 (1995).
- [13] E.C.Grose, H.R.Judy, H.T.Richard, M.Jaskot; Gluthatione peroxide and gluthationetransferase activity in rat lung and liver following cadmium inhalation. *Toxicology*, 44, 171-180 (1987).
- [14] H.Kawatsu, Y.Nakanishi, H.Takeda; Méthemoglobin determination in cell blood. Ed. Nip Sciences, 9-14 (1987).
- [15] G.Kazantziz, Cadmium: A review. *Adv.Mod.Environ. Toxicol*, 11, 127-143 (1987).
- [16] K.H.M.Kwan, S.Smith; Some aspects of the kinetics of cadmium and thallium uptake by friends of *Lema minor*, (Ed) *New Phytol*, 91 (1991).
- [17] G.F.Leong, R.L.Pessotti, R.W.Braver; Liver function in regenerating rat liver. CrPO₄ colloid uptake and bile flow. *Am.J.Physiol*, 197, 880-886 (1959).

- [18] P.Meyer; Physiologiehumaine. Ed.Flammarion Med Sciences, 683-818-820 (1983).
- [19] NIAID; The Rabbit in Immunology & Infectious Disease Research. National institute of allergy and infectious diseases. www.niaid.nih.gov, (2005).
- [20] J.Perry, M.Giroux, Y.Rouch, P.Galeau, P.Puig; Modifications biochimiques observes chez dse ouvriers travaillant en atmosphere riche en derivesoxygènes et hydrogènes d'azote. Ed.Anth.Mal.Prof, 373-382 (1987).
- [21] E.Prigge; Treshold levels of cadmium for soil respiration and growth of sping wheat and difficulties with their determination. Ed.Boil Fertil Soils, 152-157 (1978).
- [22] F.Princi; A study of industrial exposure to cadmium. J.Ind.Hyg.Toxicol, 29, 315-320 (1947).
- [23] RRagopathy, PH.D.Nasa; Spacecraft water exposure guidelines for selected contaminants. Ed.National academies press, 167 (2007).
- [24] R.Rouabhi, H.Berrebbah, M.R.Djebbar Growth, Chitin and respiratory metabolism of *Tetrahymenapyriformis* Exposed to the insecticide Novaluron. Am-Euras. J.Agric. & Environ.Sci. 3(6), 873-881 (2008).
- [25] D.A.Scicchitano, A.E.Pegg; Inhibition of O-alkylguanine-DNA alkyl transferase by metals. Mutat.Res, 192, 207-210 (1987).
- [26] Mark A.Suckow, David W.Brammer, Howard G.Rush, Clarence E.Chrisp; Biology and Diseases of Rabbits, Chapter 9 in Laboratory Animal Medicine, 2nd Edition, James G.Fox et al., (Ed); New York: Academic Press, (2002).
- [27] Mark A.Suckow, Fred Douglas; The Laboratory Rabbit, a volume in the Laboratory Animal Pocket Reference Series Boca Raton: CRC Press, (1997).
- [28] S.L.Sutton; Woodlice. Pergamon Press oxford, (1980).
- [29] C.Tseng, F.W.Leng, E.E.Chang, Tung.Yih-Shih; Studies on chronic cadmium intoxication. J.Chin.Biochem. Soc, 17, 32-41 (1988).
- [30] L.D.Tyler, M.Mcbride; Influence of Ca pH and humic acid on cadmium uptake. Ed.Plant Soit, 252-262 (1982).
- [31] B.L.Valee, D.D.Ulmer; Effect of cadmium on mitochondrial oxidative phosphorylation. Ann.Rev.Biochem, 14, 91-12 (1972).
- [32] A.Veysseyre; Dépôts de métaux lourds sur le maiteau neigeux.Ed.MASSON, 287 (2000).
- [33] G.J.Wagner; Accumulation of cadmium in corp plants and its consequence to human health. Ed.AdvAgron, 173 (1993).
- [34] J.M.Wells, D.H.Brown; Ionic control of intacellular and extracelllular Cd uptake by the mass *Rhytidiadelphus squarrosus*. Ed.Newphytol, 541-553 (1990).
- [35] G.H.Xiao, J.Y.Lui; Inhibition of amino acid in corporation of microsomes by cadmium, mercury and lead. HuanjingKexueXuebao, 7, 333-338 (1987).
- [36] L.B.Zoghlami, W.Djebali, M.H.Ghorbel; Modifications physiologiques et structurales induites par l'interaction cadmium-calcium chez la tomate (*Lycopersionexulentum*). Ed.Elsevier, 705-709 (2006).