Al-Kufa University Journal for Biology / VOL.8/ NO.2/ Year: 2016
Print ISSN: 2073-8854 & Online ISSN: 2311-6544

Hepatoprotective Effect of Matricaria chamomilla Hot Aqueous Extract Against Methomyl 90%- Induced Hepatotoxicity In Mice

Ms.c Jenan Mahdi Chani(1) Ms.c Noor Zuhair Jasim(2)

[(1,2)University Of Kufa College Of Science, Department Of Biology]

Abstract

The effect of orally feeding of hot liquid extract of (*Matricaria chamomilla* flowers) on hepatotoxicity induced to male mice by methomyl ,S-methyl-1-N-[(methylcarbamoyl)oxy]thioacetimidate (IUPAC) was investigated for(30 days). These effects could be explored by measuring any changings in the weight of both body and liver.Histopathological examination of liver has been performed also along with aspartate aminotransferase(ALAT) and alanine aminotransferase (ASAT) levels in serum.

The results showed that the poisoned group treated with pesticide by using two doses of hot aqueous extract of chamomile (5 and 7) mg / kg of body weight have shown a marked improvement of the symptoms of pesticide poisoning, compared with the positive control group of the pesticide and that the dose of the methomyl 90% led to the toxic effects appeared by decreasing in body weights in mice and noticeable changes in liver tissue accompanied by increasing in its weight.There was significant increasing in the level of liver enzymes (ASAT, ALAT), Histopathological examination of liver sections of mice administered *Matricaria chamomilla* hot aqueous extract demonstrated reduction of damaged liver tissue induced by methomyl 90%.Current study reveals that hot aqueous extract of *Matricaria chamomilla* promises protection for hepatocytes against methomyl 90%- hepatotoxic mice induction. Finally,this study recommends that intake of hot aqueous extract of *Matricaria chamomilla* could be useful for liver disorders.

Key words:methomyl insecticide, chamomile,liver,toxicity,remediation , mice.

Introduction

It is a fact that chemicals used for agricultural benefits can enter the food chain and produce a number of disorders in both human and animals (1)**.**Haddad and Winchester (1983) reported that Methomyl90% is toxic to humans since they can interfere with different vital mechanisms(2). This pesticide is known to be a carbomic acid synthetic derivative that is used for insect arbitration (3)**.**

It is considered that carbamate has been used worldwide due to it's highly effects; however, it is considered category-I toxicity pesticide (4). It has been reported that the metabolite of methomyl is considered an oncogenes. These oncogenes would be metabolite in animal tissues (5). Agha and his colleagues showed that there was acute poisonings among people who suffered methomyl effect (6).It is a fact that Chamomile contains a large number of active compounds such as crucial oil and flavonoid which usually used for an anti-inflammatory of skin and mucosa, antiseptic, and antispasmodic (7). Besides, the essential oil could be essential for treating the irritation of the respiratory tract (8). The parameters of our study have been included biochemical and histopathological changes of hepatotoxicity against methomyl 90% induced in male mice.

Materials and Methods

- *Matricaria chamomilla*.

Matricaria chamomilla is one of the family Asteraceae (7). Flowers used for this study have been purchased from (Najaf city, Iraq).

-Preparation of (*Matricaria chamomilla* hot aqueous extract)

The maceration methods (9)and(10) have been used with (distilled water) to extract the effective components of chamomile. The chamomile has been prepared in diverse concentrations by putting boiled distilled water to the dry powdered plant material with different concentrations, which are expressed in weighted and volumetric concentrations (w/v) . The weight of (5 and 7 g) of powdered chamomile is separated in individual glass, and then wet with 100 ml of distilled water and left in a hot place for (about 30 – 60) minutes with constant vibration. Then, we nominate the extracted by nomination method to get pure extracted free of impurities. The extracted has been kept in a dim sealed glass inside a refrigerator until we can use it .

-Insecticide

A procedure described by Baron was used as a reference in this study, and it is as follows:

Oral doses of methomyl have been given in distilled water to mice according to the body weight.These doses were calculated to be10 mg/ kg body wt (4). Control group were received distilled water only for 30 days.

-Experimental design

(24) male of Albinomice weighing (25-30)g and three months old were kept in (animal house at Faculty of science / University of Kufa/ Iraq), and kept in controlled environment of (22-25 °C). Commercial food (pellets) and tap water were provided to animals *ad libitum*., During the dosage period, mice were weighted every week for detecting the weight of body change. Then tested mice divided into four groups which include 6 mice.

Group 1: (Control): Food pellets and tap water were given to control mice group. This group is considered as a negative control.

Group 2: positive control were treated with a dosage of (5 mg/kg) methomyl 90%according to the body weight.

Group 3: given combination of *Matricaria chamomilla* extract(5 mg/kg with the doses of the mehomyl (5 mg/kg) according to the body weight

Group 4: given combination of *Matricaria chamomilla* extract(7 mg/kg b.wt) with mehomyl doses for body weight

Mice weights were recorded before the start and end of the experiment to measure the changes in the rate of body weight.

-Blood Samples collection

Blood samples were collected from treated mice then they were anesthetized at the end of experiment by using a mixture of ketamine and xylazine i.m. (11). The liver was weight then, removed, cleaned and fixed immediately in (10%formalin solution)for later histological preparation. 3000 rpm/ 15 minute centrifugation was used for serum collection. Serum then was kept at(-80°C).

- Analysis of serum biochemically

Spectrophotometer(USA) was used according to a method described by Tietz. The Kit used was ACCENT 200-ALAT ,ASAT ENZYME KIT/ POLAND.

- Statistical Analysis

Analysis of data was performed by using statistical Package for Social Science (SPSS) system/ version 17. Standard error was used for statistical means. The test (ANOVA) was used for this purpose.

Results

Table1: The effect of Chamomile hot aqueous extract oral administration (Mix_5& Mix_7mg/kg b.wt.) after 30 days against methomyl (5 mg/kg) .

Groups	Body weight (g) M±SE		T
	Initial	Final	
$Methomyl_5$	29.3 ±0.7	28.7±0.4	**0.52**
Mix_5	31±1.5	28.3±1.4	**0.26**
Mix_7	26.7±0.9	27.4±2.4	**0.47**

This table was show decrease in body weight of methomyl group compared with the ($mix_{5,7}$mg/kg b.wt.) but there was no significance.

Table2:Theeffect of methomyl oral administration (5gm/kg)on (body weight and liver in mice .

Groups	Body weight (g) M±SE		Liver M±E
	Initial	Final	
Control	27.3±1.7	29±1.5	1.3±0.2
$Methomyl_5$	29.3±0.7	28.7±0.4	1.7±0.1
T	0.16	0.84	0.1

This table elucidated significant decrease in the weight of the mice body compared with the control, however no significant $p<0.05$ was observed. It is noticed that the liver weight of the groups treated with insecticide was increased , the significance was as above

Table3: The effect of Mix $_5$oral administration on body weight and liver with methomyl in mice.

Groups	Body weight (g) M±SE		Liver
	Initial	Final	
Methomyl	29.3±0.7	28.7±0.4	1.7±0.1
Mix $_5$	31± 1.5	28.3±1.4	1.7±0.4
T	0.37	0.82	0.86

This table was appeared there was a decreasing in body weight of Mix 5 group compared with methomyl groups, but the result was non-significant *P<0.05* , while the liver weight of mixed group was increased compared with the methomyl , also was non- significant.

Table 4: The effect of Mix $_7$oral administration on body weight and liver with methomyl in mice.

	Body weight (g) M±SE		Liver
Groups	**Initial**	**Final**	
Methomyl	29.3±0.7	28.7±0.4	1.7±0.1
Mix $_7$	26.7±0.9	27.4±2.4	1.2±0.2
T	0.07	0.16	0.05*

*=Significant difference at level of <0.05

This table was show increasing in body weight of Mix 7 group compared with methomyl group, but non-significant *P<0.05*, while the liver weight of mixed group was significantly decreased compared with the methomyl group.

Table5: The effects of methomyl and Chamomile hot aqueous extract oral administration on serum ALAT of mice.

Groups	R1	R2	R3	mean±SE
Control	30	29	32	**30.33±0.88**
Methomyl	59	59	50	**56.00±3.00a**
Mix $_5$	47	47	50	**48.00±1.00b**
Mix$_7$	**41**	**41**	**36**	**39.33±1.66 c**

a= significantly different *P<0.05* between methomyl and control group

b= significantly different P<0.05 between mix $_5$ and methomyl group

c=significant difference P<0.05 between mix $_5$and mix $_7$ group.

This table was explain there was significantly increasing in the level of ALAT in methomyl group following by gradual significant decreasing in the level of same enzyme when comparison occur among studied groups

Table 6:The Effectiveness of orally feeding of methomyl and Chamomile hot liquid extract on serum ASAT of mice compared to each other.

Groups	R1	R2	R3	mean±SE
Control	144	178	198	**173.33±1567**
Methomyl	271	144	216	**210.3±37.5a**
Mix $_5$	264	452	264	**326.7±63.8b**
Mix 7	**433**	**281**	**321**	**345±46.4c**

a:When comparing between methomyl and control, the significant difference was $P<0.05$.

b:When comparing between mix5 and methomyl,the Significant difference was

$P< 0.05$.

c:When comparing between mix7 and mix5, the Significant difference was

$P< 0.05$.

This table was show there was significantly and gradually increasing an the level of ASAT in first three studied groups followed by strong elevated in the level of same enzyme when comparison occur among studied groups

Histological Study

The histology of mice liver was treated with methomyl for a month demonstrated that sinusoids between hypertrophied hepatocytes , distention of central vein,the cytoplasm has many vacuole and the hepatocytes lossan arrangement (figure 2).Liver of control group reveled normal hepatocytes[centrally located nuclei] with arrangement radially of hepatic cords around the central vein (figure1).

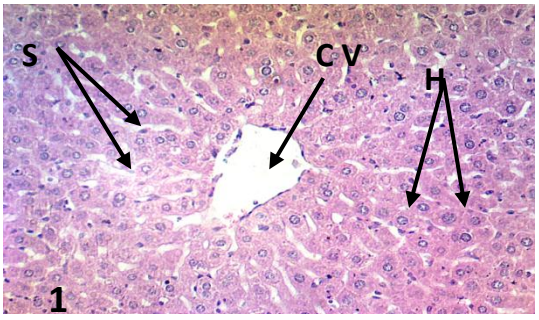

Figure1: Liver hepatocyte of control group showing: normal hepatocytes(H), central vein (CV), and sinusoids (S). H & E(200X).

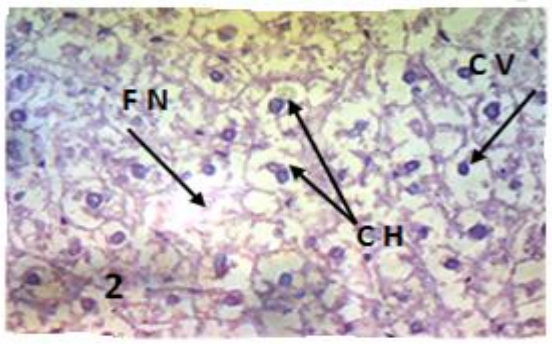

Figure 2: Liver section of methomyl group showing : cytoplasmic vaculation (C V),focal necrosis (F N) and cellular hypertrophy (C H) H & E(400X).

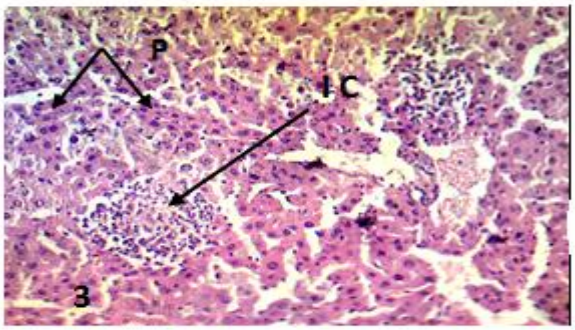

Figure 3: Liver section of methomyl group showing:diffusion inflammatory cells (I C) ,pyknosis (P) in some cells. H & E(200X).

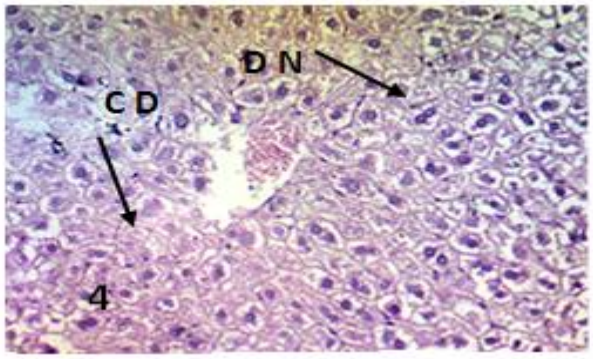

Figure 4: Liver section of methomyl group showing : cellular degeneration(CD) diploid nuclei (D N). H & E(200X) .

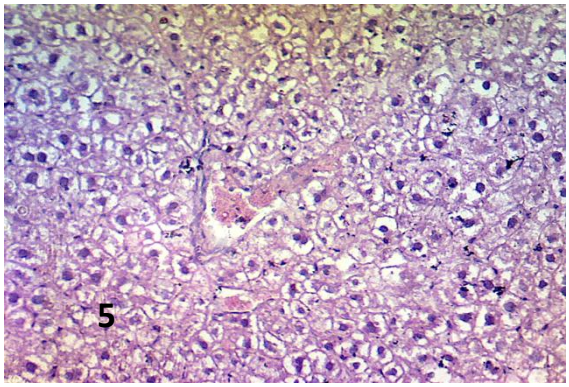

Figure 5: Liver section of Mix5 (methomyl & chamomile) 5 gm / kg b.wt.) showing : moderate changes in histological architecture . H &E(200X) .

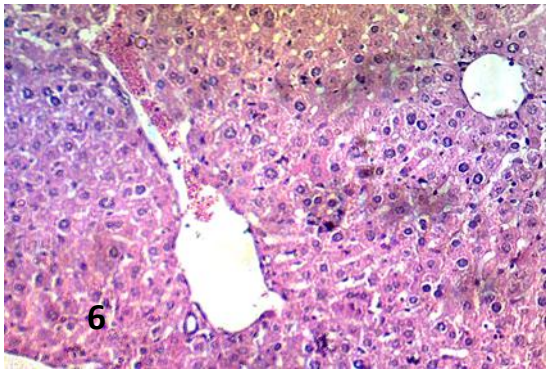

Figure 6: Liver section of Mix7 (methomyl&chamomile 7 gm / kg b.wt.) showing: less changes and normal hepatic structure . H &E (200X).

Discussion

Our study reported the effect of *M. chamomilla* hepatotoxic mice induced by methomyl.The current result disclosure that oral administration of *M. chamomilla* hot aqueous extract given together with methomyl (5mg /kg b.wt.) (table 1) to mice could increase their weight as with to mice received only (5 mg\kg b.wt.) of methomyl.this result is agreed with (13) who reported that treated mice with *M. chamomilla* hot extract in their study gained body weight when were given paractamol-intoxication. The reason of gaining weight was because the content of flavonoids in chamomile was high (14).Our study demonstrated that methomyl 90%oral administration to mice at dosage level (5mg/kg b.wt.) for (30days) induced decreased body weight compared with the negative control group but no significant (table 2)**.**Also, the decrease of mice weight could be due to difficulty of respiration and loss of mice appetite.Similar work was done in Occupational Health Services (15).

Results obtained in the current study reported that there was increase in the relative liver weight in mice treated with oral administration of methomyl 90%for 30 days as with the negatively control group (table 2).Undeger *et. al.*,(16)revealed similar results when their experimental animals were exposed to some pesticides. Moreover, (17) reported that the inductions of enzyme cytochrome-p 450 can the increase in liver weight.

In our current study, there was no significantly different observed in the body weight of mice liver that given *M. chamomilla* with methomyl 90% in dose of 5 mg\kg b. wt. each. Similar work was done by (13) (table 3).

Results of present study disclosure that *M. chamomilla* (7 mg/ kg) and methomyl 90% oral administration could decrease in liver weight as given to mice methomyl 90% only (table 4).It is studied that the administration of *M. chamomilla* hot extract decrease the liver weight in chamomile given asto negative group paracetamol treated(13).

Our result disclosure that administration of methomyl 90% at dose (5 mg\kg) to mice for (30 days) significant (p<0.05) increasing of AST and ALT as with the negatively control mice, this finding agreed with (18). (table 5, 6).

It is demonstrated that serum ALAT and ASAT are considered to be sensitive markers employed in the diagnosis of hepatotoxicity (19).These enzymes could be used along with other enzymes to monitor liver disorders (19). The concentration of these enzymes increased in blood stream when any organ damaged in the body (19). It is reported in literature that the administration of methomyl can increase ALAT and ASAT (20)and (21).The activity of serum transaminase could also be increased in the bloodstream, suggesting liver injury (22, 23, and 24) and cell damage. It is demonstrated that parathion and cadmium can induce high level of enzymes (ALAT and ASAT) (25).

In molecular biology term, the high level of serum enzymes could be due to gene expression(26).

From the histology point of view, there was extension of central vein and sinusoids within cytoplasm of mice treated liver with 5mg/kg methomyl for(30 days)reported that (figure 3).This finding agreed with the (27).The control mice liver revealed normal hepatocyte (figure 1and 2).

It is reported that high level of methomyl can cause cytoplasmic vacuolization hyalization,hypertrophy of hepatocytes ,Destination of central vein ,pycnotic nuclei ,moreover loss of radial arrangement of hepatocytes (28). In addition, necrotic condition of liver cells seen in figure 4 was due to the cell inflammatory response (29) (figure4). Moreover, it is demonstrated that methomyl carbamate can cause necrosis of focal hepatocellular (30) (31) (32) (figure 3).

It is reviewed from the literature that other material such as carbendazim and carbosulfan can alter the histogical properties in mice liver when exposed to them (33) and (34)

Finally, our results showed that the continuous exposure of carbamate acid has effect on mouse liver.

Conclusion:

It could be concluded that feeding orally of *M.chamomile* hot aqueous extract to methomyl 90% insecticide -intoxicated mice for 30 days improves body weight and promise protection for hepatocytes and has an antioxidant effects and also induce degenerative changes seen in liver tissues.The present study recommends that intake of *Matricaria chamomile* flowers is useful for liver disorders.

REFRENCE

1- Raseir, G., Toppari, J., Parent, A. and Bourguignor, J. (2006) Female sexual maturation and reproduction after prepubertal exposure to estrogens and endocrine disrupting chemicals: A review of rodent and human data, Mol. Cell Endocrinol., vol. 254: 187–201.

2- Haddad LM& Winchester JF (1983) Clinical management of poisoning and drug overdose. WB Saunders &Co., Philadelphia, London, Toronto: 364-373.

3- Abd Rabou A & Al-Agha M (1998) Environmental awareness in handling and application of pesticides among farmers in Rafah governorate-Gaza Strip. The Vth International HCH and Pesticide Forum, Bilbao, Spain. June, 25-27, 1998.

4- Baron R. L. (1991) Carbamate insecticides, In Handbook of Pesticide Toxicology – Vol 3, Hayes WJ., Laws E.R. (Eds).San Diego, Calif, Academic Press, New York.pp 1125-90.

5- EPA. (1996) Drinking Water Regulations and Health Advisories,USEPA, 822-B-96-002, Washington, DC

6- Agha A., Dib S., Al-Hakami M., Abdulhadi Ali M. (2009) The IntrnetJrnl of Toxicol, 7:1.

7-Singh, O., Khanam, Z., Misra, N. and Srivastava, M. (2011) Chamomile (matricaria chamomilla l.): An overview, Phcog. Rev., vol. 5: 82-95.

8- Wang, Y., Tang, H., Nicholson, J., Hylands, P., Sampson, J. and Holmes, E. (2005) Metabonic strategy for the detection of the metabolic effects of Chamomile(matricaria recutita l.) ingestion, J. Agricultural and Food Chem., vol. 53: 191-196.

9- Gruenwald, J.; Brendler, T. and Jaenick, C. (1998). PDRfor herbal medicine .1stEd. economics company, Inc pp: 981-982.

10- Newall, C.A.; Anderso, A. and Phillipson, J.D.1996).Herbal medicine: A guide for Health care professionals. The pharmaceutical press. London pp: 49-70.

11- Schiller, N.K. and McNamara, D.B. (1999). Balloon catheter vascular injury of the alloxan- induced diabetic rabbit: The role of insulin-like growth factor-1. Molecular and Cellular Biochemistry, 202:159-167.

12-Tietz, N. W. (2006) Textbook of clinical chemistry and molecular diagnostics, Edited by: C.A. Burtis, E.R. Ashwood and D.E. Bruns, CA: Elsevier Saunders.

13-Gupta, A. and Misra, N. (2006) Hepatoprotective activity of aqueous ethanolic extract of Matricaria chamomilla in paracetamol intoxicated albino rats, American Journal of Pharmacology and Toxicology, vol. 1: 17-20

14-Bradberry, S. M., Proudfoot, A. T. and Vale, J. A. (2004) Poisoning due to chlorophenoxy herbicides, Toxicol. Rev., vol. 23: 65-73.

15-Occupational Health Services, Inc. 1986. Material safety data sheet .Secaucus,NJ: OHS.Inc.

16-Undeger, U., Institoris, L., Siroki, O., Nehez, M. and Desi, I. (2000) Simultaneous geno- and immunotoxicological investigations for early detection of organophosphate toxicity in rats, Ecotoxicol. Environ. Safe., vol. 45: 43–48.

17-Amacher, D., Schomaker, S. and Burkhardart, J. (1998) The relationship among microsomal enzyme induction, liver weight and histological change in rat toxicology studies, Food Chem. Toxicol., vol. 36: 831–839

18- Troudi, A., Amara, I., Samet, A. and Zeghal, N. (2012) Oxidative stress induced by 2, 4-phenoxyacetic acid in liver of female rats and their progeny:Biochemical and histopathological studies, Environmental Toxicology, vol. 27: 137-145.

19- Purcell,G.V.; Behenna, D.B.; Walsh, P.R.; Alanine aminotransferase and aspartate aminotransferase measurements with two automated analyzers, SMAC and the ABA-100, Compared. Clin.Chem. 1979, 25, 780-782.

20- Guilhermino L, Soares AMVM, Caenalho AP& Lopes MC (1998) Effects of cadmium and parathion exposure on hematology and blood biochemistry of adult male rats. Bulletin of Environmental Contamination &Toxicology 60: 52-59

21-Ashour A (1999) Comparative study on the effect of some insecticides administration on protein content and some enzymes of rabbit's serum. Annual Review for Arts, Science & Education, Faculty of Women, Ain Shams University: Science section 20: 72-87

22-Rigon AR, Reis M &Takahashi RN (1994) Effects of carbaryl on some dopaminergic behaviours in rats.General Pharmacology 25(6): 1263-1267

23-Kutlu S, Colakoglu N, Halifeoglu I, Sanadal S, Seyran AD, Aydin M & Yilmaz B (2005) Comparative evaluation of hepatotoxic and nephrotoxic effect of aroclors 1221 and 1254 in female rats. Cell Biochemistry & Function 25(2): 167-172

24- Dewan A, Bhatnager VK, Mathur M, Chakama T, Kashyap R, Sadhu HG, Sinha SK& Saiyed HN (2004) Repeated episodes of endosulphan poisoning. ClinicalToxicology 42(4): 363-369

25- Funakoshi T, Ohta O, Shimada H& Kojima S (1995) Effects of dithiocarbamates and cadmium on enzymatic activities in liver, kidney and blood of mice. Toxicology Letters 78: 183-188

26- Friedman LS, Brautbar N, Barach P, Wolfe A& Richter ED (2003)Creatine phosphate kinase elevations signaling muscle damage following exposures to anticholin esterases: 2 sentinel patients. Archives of Environmental Health 58(3): 167-71

27-Yoon J.Y., Oh S.H., Yoo S.M., Lee S.J., Lee H.S., Choi S.J., Moon C.K., Lee B.H. (2001) Toxicolog, 169: 153-161

28- Raffray M., Cohen G.M. (1997) *PharmacolTher*, 75: 153-177.

29-Wyllie A.H. (1980) *Nature*, 284: 555-556.

30-Quest J.A., Chan P.C., Crawford D., Kanagalingam K.K., Hall W.C. (1987) Fundam Appl Toxicol, 8(3): 389-99.

31-Landon E.J., Naukam R.J., Rama Sastry B.V. (1986) Biochem Pharmacol, 35: 697-705.

32-Diez-fernandez C., Bosca L., Fernandezsimon L., Alvarez A., Cascales M. (1993) Hepatology, 18: 912-918.

33-McCarrol N.E., Protzel A., Ioannou Y., Frank Stack H.F., Jackson M.A., Waters M.D., Dearfield K.L. (2002) Mutat Res, 512(1): 1-35.

34-Ksheerasagar R.L., Kaliwal B.B. (2006) Caspian J Env Sci, 4(1): 61-70.