

**Determination of some Heavy Metals in Extraction of plants by
using different solvents**

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Summary

The present study was conduct to determination of the contents of Extraction of plants leaves with heavy metals, the samples represent five *Eucalyptus camaldulensis Dehnh*, *Vicia*, *Jasminum Humile* collected from different positions at Nasiriya zone through March to April 2011 were analyzed for heavy metals. The cold extraction method including use of ethanol 95 % , Acetone, petroleum ether, chloroform and cyclohexane .Maximum levels of heavy metals in the analyzed samples were Pb, Cr, Zn ,Cu, and Fe, respectively.

Introduction

The understanding of fundamental principles and phenomena that control the transfer of trace elements in soil-plant-human chain can contribute to the protection of the environment and human health. Many books, articles, and reports have already described both fundamental and/or detail problems related to these topics.

Plant extracts represents a complex mixture containing both organic and inorganic chemical species, being trace metals one group the inorganic components present in this type of matrix. Information on trace element concentrations in Plant extracts is getting increasingly important for the clinical characterization of diseases and also to allow interferences actions during Plant extractions processing (U.S.L 2004).Trace metals have been found in different proportions in different plant crudes and consequently in their derivatives. Trace element concentrations in plants reflect, in most cases, their abundance in growth media (soil, nutrient solution, water) and in ambient air (London Lab. Services group, 2003) .

Heavy metals get into plants via adsorption which refers to binding of materials onto the surface or absorption which implies penetration of metals into the inner matrix. Both mechanisms can also occur(Lokeshwary and Chandrappa, 2006) .

In small concentrations, the traces of the heavy metals in plants or animals are not toxic(Vries et al, 2007). Lead, Chromium and copper are exceptions; they are toxic even in low concentrations(Gorcher, 1991). Monitoring of the levels of heavy metals in plants with is of interest due to their influence on animals and humans (Olubunmi and Edward, 2010).

In order to investigate how plant stress metabolism is influenced by trace element content, many analytical laboratories have recently determined the trace elements present in them. These studies have tended to focus on the separation of trace elements by many methods,

but no specific approach were found in literature regarding investigate the trace element content .One of them via the sequential extraction of substances binding trace elements in plant biomass (Daniela , 2005). Another approach for plant analysis used direct ETAAS after dissolving the samples in a suitable organic solvent. The organic solvent used 1,4-dioxane, ensures complete dissolution of the samples and a possibility to use aqueous standard solutions for calibration purposes (Karadjova et al, 1998) .

The famous method via the digestion of samples by add Nitric acid (HNO_3), Sulfuric acid (H_2SO_4) and Perchloric acid (HClO_4) (5:1:0.5). The sample boiled in acid solution in fume hood on hot plate till the digestion is complete. Thereafter, few drops of distilled water are add and allow to cool. The solutions were analyzed for the elements of interest utilizing Atomic Absorption Spectrometer AAS (Abou-Arab,2000)

In the present work we used a different organic solvents to show their ability to release ions of heavy metals to solution ,this method give idea about the best solvent which can be use to dissolve the organic matter and release the ion ,that's give a specific investigation of trace element by AAS.

Experimental

Samples Collection: A total of 25 samples, which represent five different types of leaves plants through period between March and

April 2011 and each agricultural practices, were collected from different positions of Nasiriya City southern of Iraq. The collected samples were kept in plastic bags until their analysis. The various collected samples (scientific name, No. of collected samples) are presented in Table (1).

Material of heavy metals, that is, lead (Pb), chromium (Cr), zinc (Zn), copper (Cu), and iron (Fe), were provided by Merck (Germany).

Procedure : 25 ml of the following organic solvents (ethanol 95 % ,Acetone , petroleum ether , chloroform ,cyclohexan individually) were add to 0.5 gram of samples in 100 ml flask . The solutions were shaking by (shaker Lap Companion HR 120) Then filter the extract with filter paper (Whatmann No. 42) then filtrate were collected in labeled plastic bottles. The solutions were analyzed for the elements of interest utilizing Atomic Absorption Spectrometer PG 990 with suitable hollow cathode lamps. The percentages of different elements in these samples were determined by the corresponding standard calibration curves obtained by using standard AR grade solutions of the elements i.e. Cu^{+2} , Cr^{+3} , Zn^{+2} , Fe^{+3} , and Pb^{+2} .

Statistical analysis

Results were analyzed statistically using analysis of variance (ANOVA) and test less significant difference among the extracts in the solvent and among the solvents in extract, according to the statistical system(SPSS) as well as the extracted average value and the value of standard deviation(STD) of the values conc. of metals for all extracts in the solvent and for the all solvents in extract, as well as calculated the

correlation coefficient among all values conc. of metals within the Confidence limits 95%.

Table (1). The various collected samples (scientific name, number)

Scientific Name	No. of collected samples
<i>Nerium oleander</i>	5
<i>Conocarpus lancifolins</i>	5
<i>Eucalyptus camaldulensis Dehnh</i>	5
<i>Vicia</i>	5
<i>Jasminum Humile</i>	5

Results and discussion

Generally , in all extracts, the Fe values were high under all organic solvent , that's natural result to concentrations of iron ion in most plant especially leaves in chlorophylls , the result conformable to its concentrations in plant ash 1600 ppm (Reichman and Parker,2005) . The levels of Zn were followed levels of Fe in same plants , this result consider normal due to Zn is essential to growth of plants. The levels of Pb were followed levels of Zn in same plants , this result consider up normal can be attributed to pollution volume of soil ,water and air in study zone (Reimann and Caritat ,1998) . So concentrations of Pb in natural water was 0.06 – 120 $\mu\text{g} \setminus \text{L}$, in sea water 0.03 – 13 $\mu\text{g} \setminus \text{L}$ and plant ash 30 ppm (Reichman and Parker,2005) .The fourth level was Cr in most extract under all organic solvent were the lowest because low concentration of their in soil and water generally .

The last was Cr which consider a basic content in most plants , so the high level from it can be due to using of fungaceous pesticides and mostly useful copper oxides . concentrations of trace elements in plants are often positively correlated with the abundance of these elements in growth media. In addition to soil is the main source of trace elements for plants both as micronutrients and as pollutants.

Table (2) Approximate common concentrations of trace elements in mature leaf tissues generalized for various plant species (µg/ g) according to (Kabata- Pendias and Pendias 2001)

Elements	Sufficient or normal	Excessive or toxic	Tolerable in crop plant
Pb	5-10	30-300	0.5-10
Cr	0.1-0.5	5-30	2
Cu	5-30	20-100	5-20
Fe	45-200	200-500	100
Zn	25-150	100-400	50-100

A number of studies have been carried out on trace element toxicity to plants and the results vary with each experiment and each plant. However, it can be generalized that the most toxic trace metals for both higher plants.(Prasad and Hagemeyer ,1999) summarized the main plant reactions to the toxicity of element excesses, as Cu, Pb causes changes in permeability of cell membranes and reactions of thiol group with cations .

Iron contents of plants vary greatly and indicate the impact of soil and climatic conditions. Mean Fe concentrations in all plants at different solvents range from 14 to 122, being the lowest for Australia

Hungary and New Zealand. Mean Fe amounts in clover vary from 117 to 400 $\mu\text{g} / \text{g}$.

The nutritional requirement of grazing animals is usually met at the Fe contents ranging from around 50 to 100 $\mu\text{g} / \text{g}$. Contents of Fe in moss growing in Norway vary highly, from 120 to 21 000 $\mu\text{g} / \text{g}$ and average 660 $\mu\text{g} / \text{g}$, suggesting the impact of atmospheric deposition. (Berg and Steinnes ,1997) Results of the research of(Nieminen *et al*,2004) indicated that Fe from aerial deposition dominates (Fe in pine needles: 883 $\mu\text{g} / \text{g}$) over root uptake pathway of this metal (Fe in pine needles: 69 $\mu\text{g} / \text{g}$).

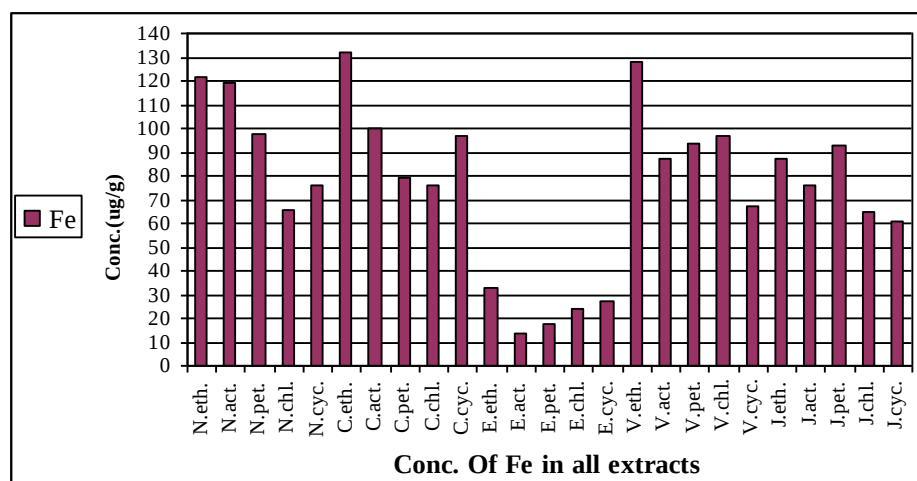


Fig. 1 : Concentrations of Fe ($\mu\text{g g}^{-1}$) in all plants

In all samples, Cr contents are fairly uniform . Contents of Cr seem to be rather stable and vary either from 2 to 47 $\mu\text{g} / \text{g}$ (Kabataand Pendias ,2001) .The highest content of Cr is in *Jasminum Humilein* petroleum ether (47 $\mu\text{g} / \text{g}$), Relatively lower amounts of Cr are found in *Nerium oleander* in Acetone (0.16 $\mu\text{g} / \text{g}$).

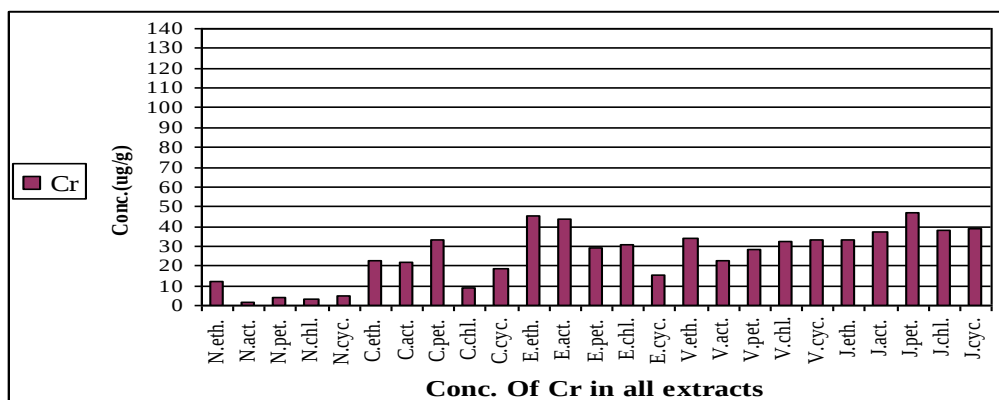


Fig. 2 : Concentrations of Cr ($\mu\text{g g}^{-1}$) in all plants

Chromium contents in moss samples from Norway, collected during the period 1990–1995, averaged $2.6 \mu\text{g/g}$, in the range of $0.21\text{--}290 \mu\text{g/g}$, across all sampling sites (Berg and Steinnes, 1997). Lichens from Wisconsin State, USA sampled during the period 1987–2001 contained Cr at the average value of $1.2 \mu\text{g/g}$, in the range from 1.1 to $1.4 \mu\text{g/g}$. These data indicated a rather stable emission of Cr to the atmosphere in these regions (Bennett and Wetmore, 2003).

Although the Zn contents of plants vary considerably, reflecting impact of different factors of the various ecosystems and of genotypes, in certain food and fodder plants its content is fairly similar (Siegert et al, 1986). Zinc deficiency in plants is generally observed when the plant contains less than $20 \mu\text{g/g}$ and toxic effects are expected when the concentration exceeds $300\text{--}400 \mu\text{g/g}$ (Boawn and Rasmussen, 1971). The mean Zn concentrations vary between 22 and $112 \mu\text{g/g}$, being the lowest in *Conocarpus* in cyclohexane and the highest in *Nerium oleander*

in ethanol. Elevated Zn contents are observed only in cassava grain from Nigeria and in rice from industrial regions of India. In vegetables, Zn also does not show any accumulation in a specific plant (Hassanin et al, 1993)

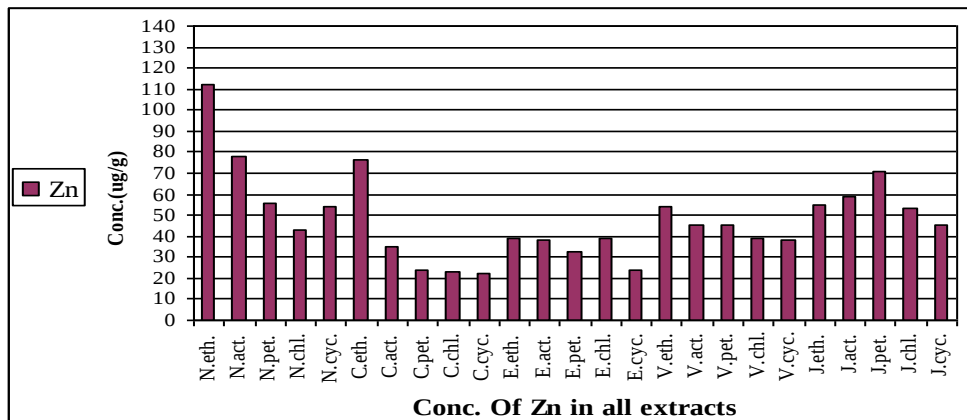


Fig. 3 : Concentrations of Zn ($\mu\text{g g}^{-1}$) in all plants

Copper is slightly mobile in plants as it is strongly bound by nitrogen and proteins. With Cu deficiency, a plant may grow normal vegetation for a short time, but there will be a failure in the redistribution of Cu from old leaves and roots causing chlorosis and tip necrosis in new leaves, known as Cu deficiency symptoms (Kabataand Pendias ,2001).

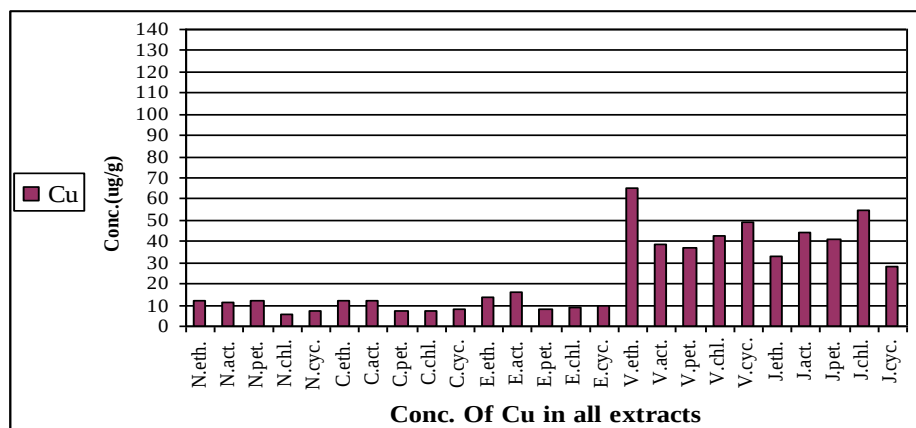


Fig. 4 : Concentrations of Cu ($\mu\text{g g}^{-1}$) in all plants

Plants growing on Cu-polluted sites tend to accumulate increased amounts of this metal, especially near industrial areas, and in soils treated with Cu-bearing herbicides (Reimann and Caritat 1998) .A number of projects have been carried out recently on Cu levels in edible plants, since about 30% of daily Cu intake by adults is from food plants(Read and Martin ,1993).

Lead in plants is inherited from both, soil and atmospheric pollution. The variation of Pb contents of plants is highly influenced by environmental factors (Sillanp and Jansson ,1992).

Lead occurs naturally in all plants, however is has not been shown yet to play any essential roles in their metabolism. There has been some discussion on this topic, and final conclusion was that if Pb would be necessary to plants it could be at the low level of 2–6 $\mu\text{g kg}^{-1}$ (Kabataand Pendias ,2001). The absorption of Pb by root is passive and thus the rate of its uptake from soils is rather low.

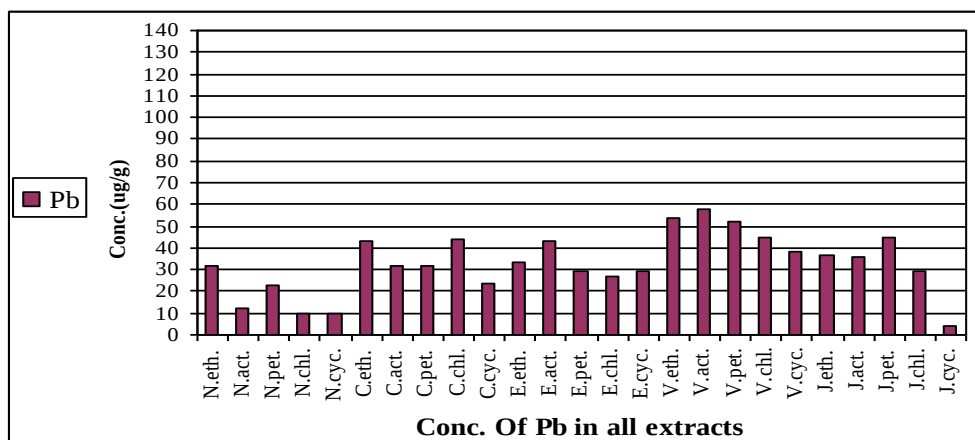


Fig. 5 : Concentrations of Pb($\mu\text{g g}^{-1}$) in all plants

Its transfer in plants is very limited and its great proportion is accumulated in roots. However, increased level of Pb in some plants has been used as a very good indicator for the geochemical prospecting (Chandra et al ,2005).

The statistical analysis showed at ($p<0.05$) significant differences among conc. of all elements of the plants extracts in all solvents that's may be attributed to effect of different solvents and nature of plant. But when compared conc. of metals in one extract by using different solvent at ($p<0.05$) showed significant differences at all tests except Cr in *Nerium oleander* in ethanol and *Vicia* in cyclohexane and chloroform .

Table(2) Fig. 1 : Concentrations of heavy metals ($\mu\text{g g}^{-1}$) in all plants and Mean $\pm$ Std. Deviation

Extracts	Symbol	Conc.(ug/g)				
		Pb	Cr	Cu	Fe	Zn
Nerium oleander in ethanol	N.eth.	32	12	12	122	112
Nerium oleander in acetone	N.act.	12	2	11	119	78
Nerium oleander in petroleum ether	N.pet.	23	4	12	98	56
Nerium oleander in chloroform	N.chl.	10	3	6	66	43
Nerium oleander in cyclohexane	N.cyc.	10	5	7	76	54
Conocarpus in ethanol	C.eth.	43	23	12	132	76
Conocarpus in acetone	C.act.	32	22	12	100	35
Conocarpus in petroleum ether	C.pet.	32	33	7	79	24
Conocarpus in chloroform	C.chl.	44	9	7	76	23
Conocarpus in cyclohexane	C.cyc.	24	19	8	97	22
Eucalyptus in ethanol	E.eth.	33	45	14	33	39
Eucalyptus in acetone	E.act.	43	44	16	14	38
Eucalyptus in petroleum ether	E.pet.	29	29	8	18	33
Eucalyptus in chloroform	E.chl.	27	31	9	24	39
Eucalyptus in cyclohexane	E.cyc.	29	15	10	27	24
Vicia in ethanol	V.eth.	54	34	65	128	54
Vicia in acetone	V.act.	58	23	39	87	45
Vicia in petroleum ether	V.pet.	52	28	37	94	45
Vicia in chloroform	V.chl.	45	32	43	97	39
Vicia in cyclohexane	V.cyc.	38	33	49	67	38
Jasminum Humilein ethanol	J.eth.	37	33	33	87	55
Jasminum Humilein acetone	J.act.	36	37	44	76	59
Jasminum Humilein petroleum ether	J.pet.	45	47	41	93	71
Jasminum Humilein chloroform	J.chl.	29	38	55	65	53
Jasminum Humilein cyclohexane	J.cyc.	4	39	28	61	45
Mean $\pm$Std.Deviation		32.8 $\pm$ 13.81	25.6 $\pm$ 13.62	23.4 $\pm$ 17.83	77.4 $\pm$ 33.24	48 $\pm$ 20.13

Also Cu in *Nerium oleander* in ethanol, petroleum ether and *Conocarpus* in ethanol and acetone so the Zn of *Eucalyptus* in ethanol, petroleum ether and chloroform. That's mean use any one of these solvent in cases above give similar results .

Also The statistical analysis showed don't found a significant positive correlation ($P < 0.05$) between Pb and all extracts but found inverse significant correlation ($p < 0.05$) with Pb and extracts of acetone and chloroform ($r = -0.638$), ($r = -0.615$) respectively and at ($p < 0.01$) inverse significant correlation for Pb in *Jasminum Humilein* cyclohexane that mean the later don't prefer to determination of Pb in *Jasminum Humilein*.

As well as the results of statistical analysis showed significant positive correlation ($P < 0.01$) ($r = 0.718$) between Zn and *Eucalyptus* in acetone , inverse significant correlation ($p < 0.05$) with Zn and *Vicia* in ethanol and ethanol ($r = -0.621$) ($r = -0.565$) respectively. For Fe statistical analysis showed significant positive correlation ($P < 0.05$) ($r = 0.64$) with *Eucalyptus* in acetone but inverse significant correlation at ($p < 0.01$) with Fe all extracts except *Jasminum Humilein* in acetone ($r = -0.956$) that mean the optimum method to determination of Fe in *Eucalyptus* conduct by acetone . The results also show significant positive correlation ($p < 0.05$) ($r = -0.522$) between Cr and *Eucalyptus* in cyclohexane but inverse significant correlation ($p < 0.05$) ($r = -0.607$) with extracts of *Jasminum Humilein* in chloroform and ($r = -0.792$) *Jasminum Humilein* in cyclohexane for Cu don't appear any significant positive correlation with all extracts but appeared inverse significant correlation with all

extracts that indicate to don't prefer use of organic solvent in the present study to determination of Cu.

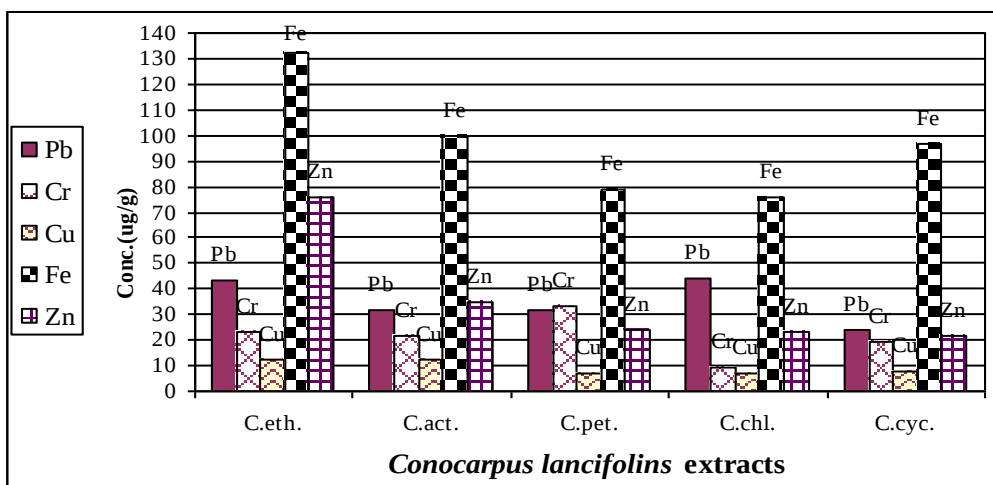


Fig. (6) Conc. of metals in *Conocarpus* extracts

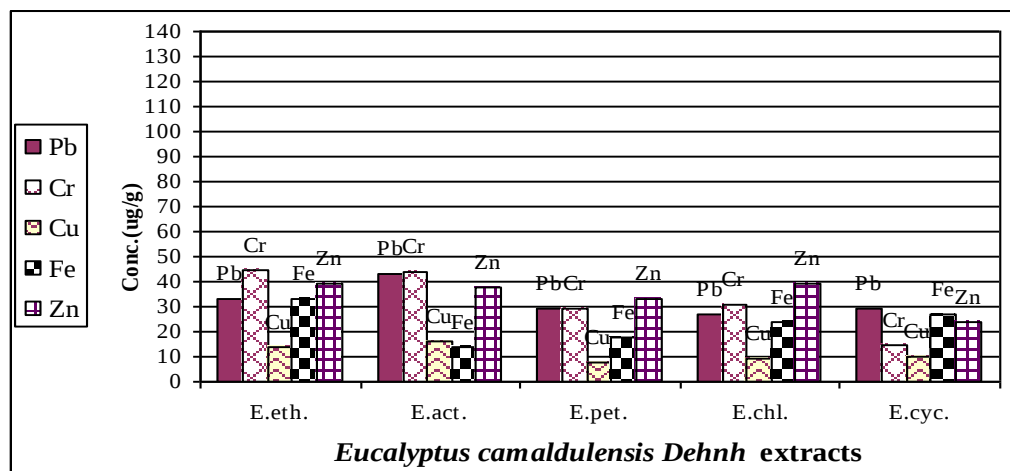


Fig. (7) Conc. of metals in *Eucalyptus* extracts

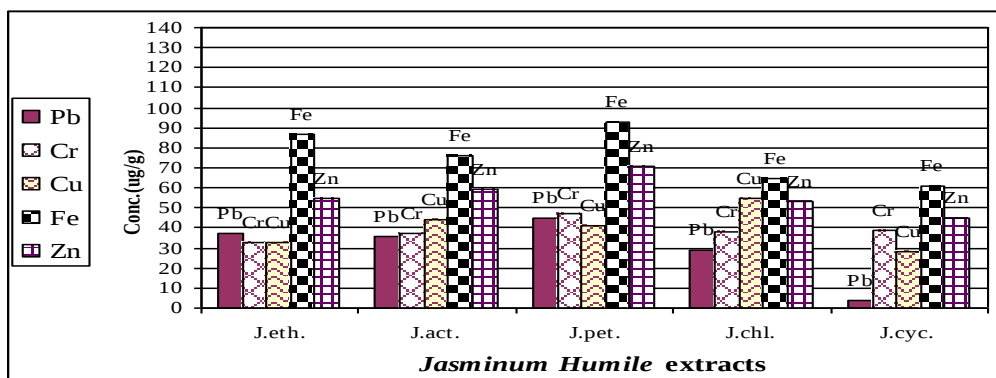


Fig. (8) Conc. of metals in *Jasminum Humile* extracts

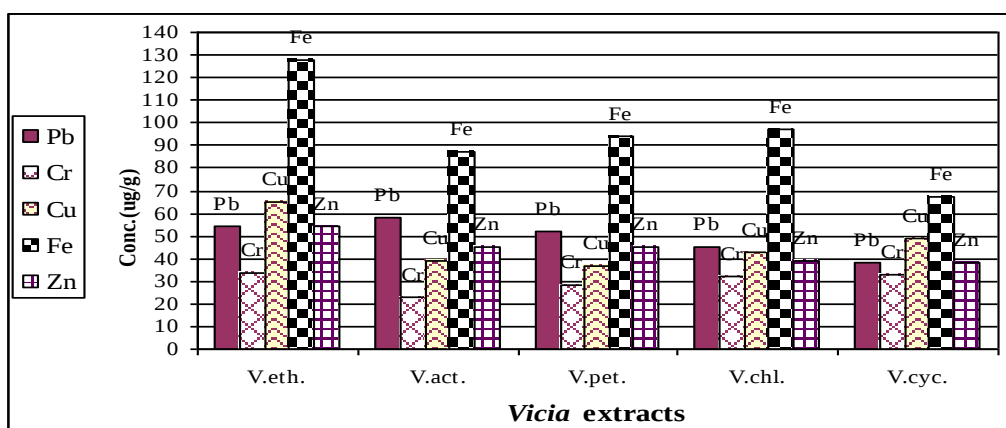


Fig. (9) Conc. of metals in *Vicia* extracts

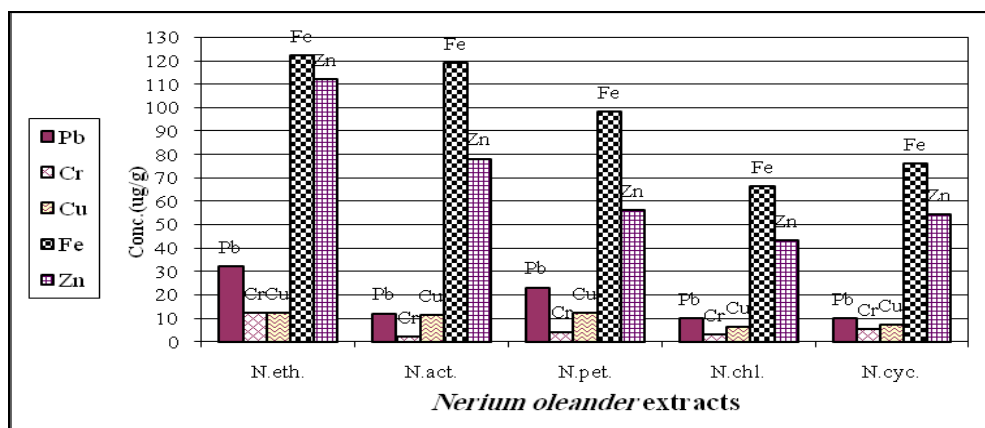


Fig. (10) Conc. of metals in *Nerium oleander* extracts

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تقدير بعض العناصر الثقيلة في مستخلصات نباتية باستخدام مذيبيات عضوية
مختلفة

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