

Determination of some heavy metals in extraction of plants by using AAS Technique

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Abstract:

The present study was conducted to determine heavy metals in the contents of aqueous and organic solvent extracts of plant leaves. Five different types of plant leaves were *Nerium oleander*, *Conocarpus lancifolius*, *Eucalyptus camaldulensis* Dehnh, *Vicia*, *Jasminum Humile* collected from different positions at Nasiriya zone were analyzed for heavy metals. The cold aqueous and organic solvent extraction method by using Atomic Absorption Spectroscopy AAS technique was used for extraction. The general main levels of heavy metals in the total analyzed samples were arranged as Fe > Zn > Pb > Cu > Cr respectively. The study showed that there is an ideal solvent to extract all the elements in the above varieties of study plants, and the results compared with standard ranges and other studies.

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 represent 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 interference actions during plant extraction 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 was found in literature regarding investigation of 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 adding Nitric acid (HNO₃), Sulfuric acid (H₂SO₄) and Perchloric acid (HClO₄) (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 added and allowed to cool. The solutions were analyzed for the elements of interest utilizing Atomic Absorption Spectrometer AAS (Abou-Arab, 2000)

In the present work water used a solvent to show their ability to release ions of heavy metals to give a specific investigation of trace element by AAS.

Experimental:

Samples Collection: A total of 25 samples, which represent five different types of plant leaves through period between March and April 2015 and each agricultural practice, 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 deionize water solvent 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 used to analyzed (ethanol , acetone , petroleum ether ,chloroform & cyclohexane) 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 $\mu\text{g.L}^{-1}$, in sea water 0.03–13 $\mu\text{g.L}^{-1}$ 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 ($\mu\text{g.g}^{-1}$) 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 and 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 $\mu\text{g.g}^{-1}$. The nutritional requirement of grazing animals is usually met at the Fe contents ranging from around 50 to 100 $\mu\text{g.g}^{-1}$.Contents of Fe in moss growing in Norway vary highly, from 120 to 21 000 $\mu\text{g.g}^{-1}$ and average 660 $\mu\text{g.g}^{-1}$, 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 $\mu\text{g.g}^{-1}$) over root uptake pathway of this metal (Fe in pine needles: 69 $\mu\text{g.g}^{-1}$).

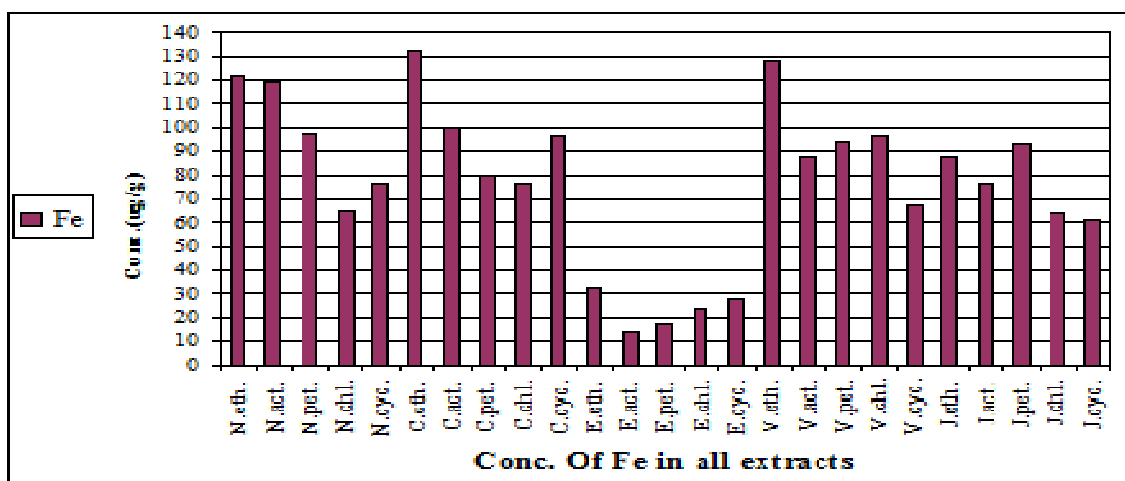


Fig. 1 : Concentrations of Fe ($\mu\text{g.g}^{-1}$) in all plants

In all samples, Cr contents are fairly uniform. Contents of Cr seem to be rather stable and vary either from 2 to 47 $\mu\text{g.g}^{-1}$ (Kabataand Pendas ,2001) .The highest content of Cr is in *Jasminum Humilein* petroleum ether (47 $\mu\text{g.g}^{-1}$), Relatively lower amounts of Cr are found in *Nerium oleander* in Acetone (0.16 $\mu\text{g.g}^{-1}$).

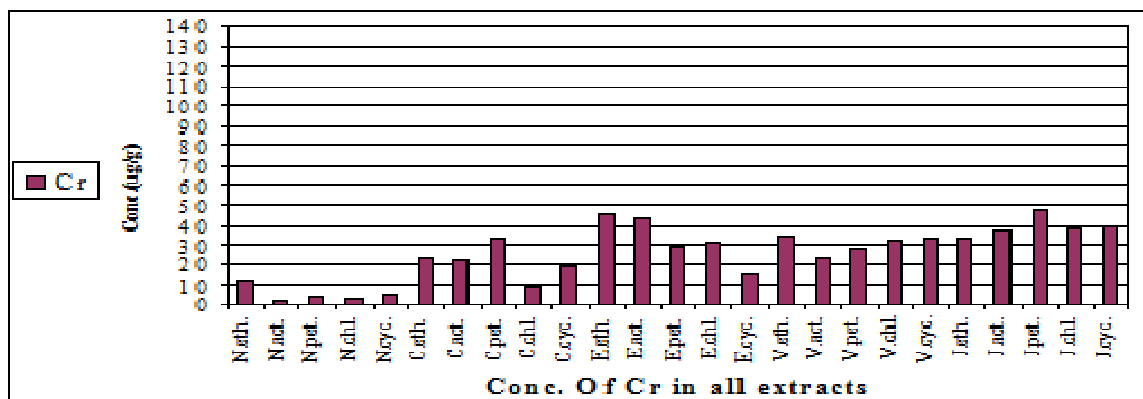


Fig. 2 : Concentrations of Cr ($\mu\text{g.g}^{-1}$) in all plants

Chromium contents in moss samples from Norway, collected during the period 1990–1995, averaged 2.6 $\mu\text{g.g}^{-1}$, in the range of 0.21–290 $\mu\text{g.g}^{-1}$, 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 $\mu\text{g.g}^{-1}$, in the range from 1.1 to 1.4 $\mu\text{g.g}^{-1}$. 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 $\mu\text{g.g}^{-1}$ and toxic effects are expected when the concentration exceeds 300–400 $\mu\text{g.g}^{-1}$ (Boawn and Rasmussen , 1971). The mean Zn concentrations vary between 22 and 112 $\mu\text{g.g}^{-1}$, 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).

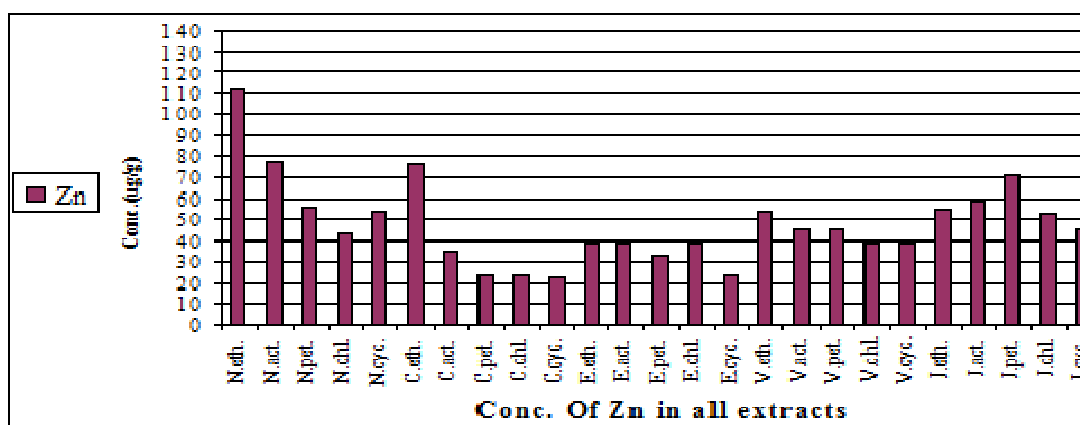


Fig. 3 : Concentrations of Zn (µg.g⁻¹) in all plants

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).

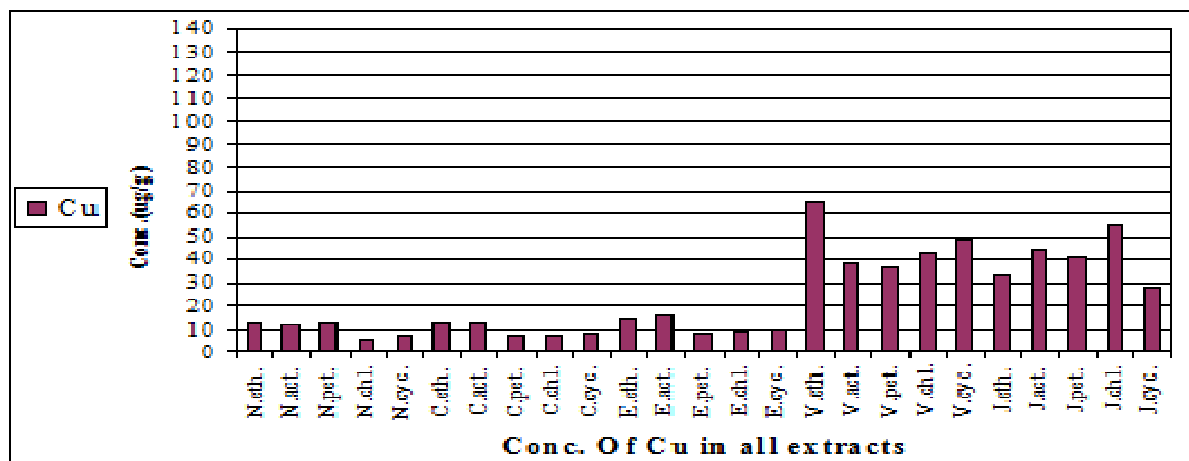


Fig. 4 : Concentrations of Cu (µg.g⁻¹) in all plants

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 µg .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.

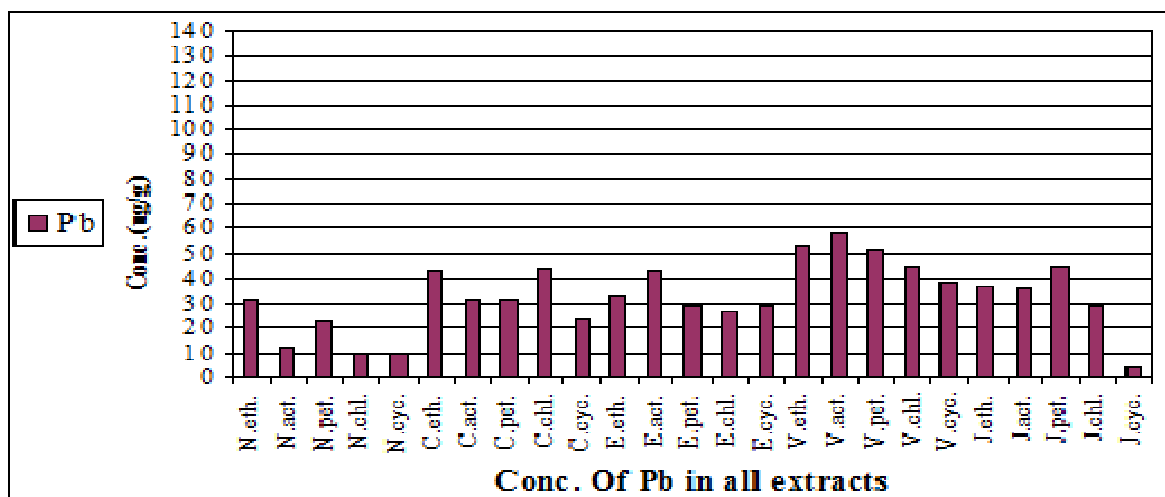


Fig. 5 : Concentrations of Pb(µg.g⁻¹) in all plants

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 concentration 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) : Concentrations of heavy metals (µg.g⁻¹) in all plants and Mean ±Std. Deviation

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

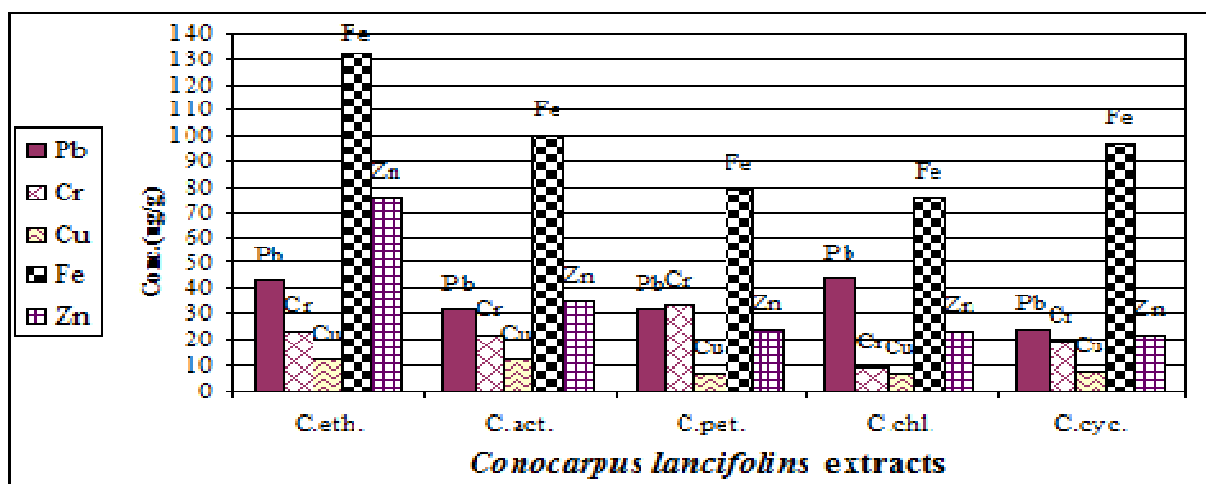


Fig. (6): Conc. of metals in *Conocarpus* extracts

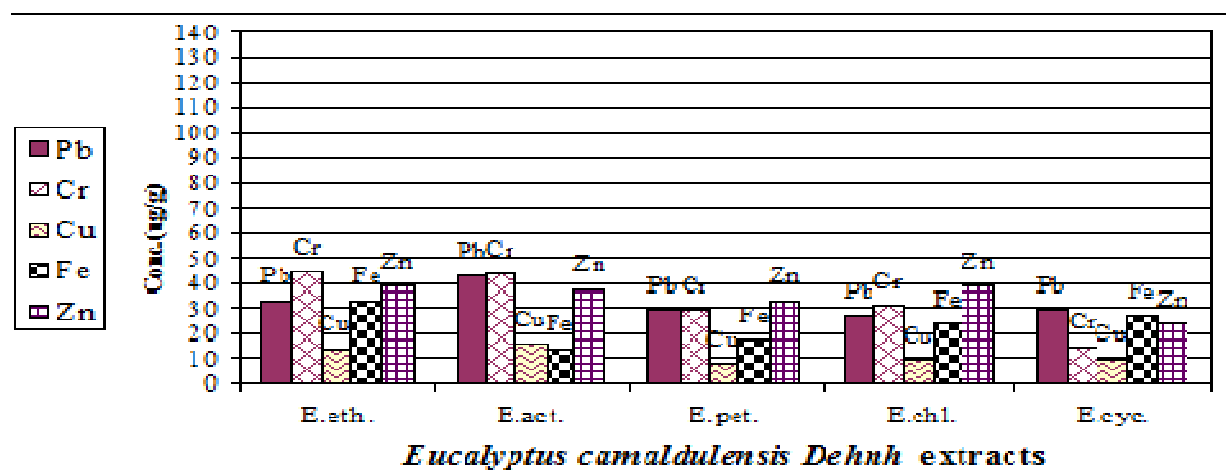


Fig. (7) :Conc. of metals in *Eucalyptus* extracts

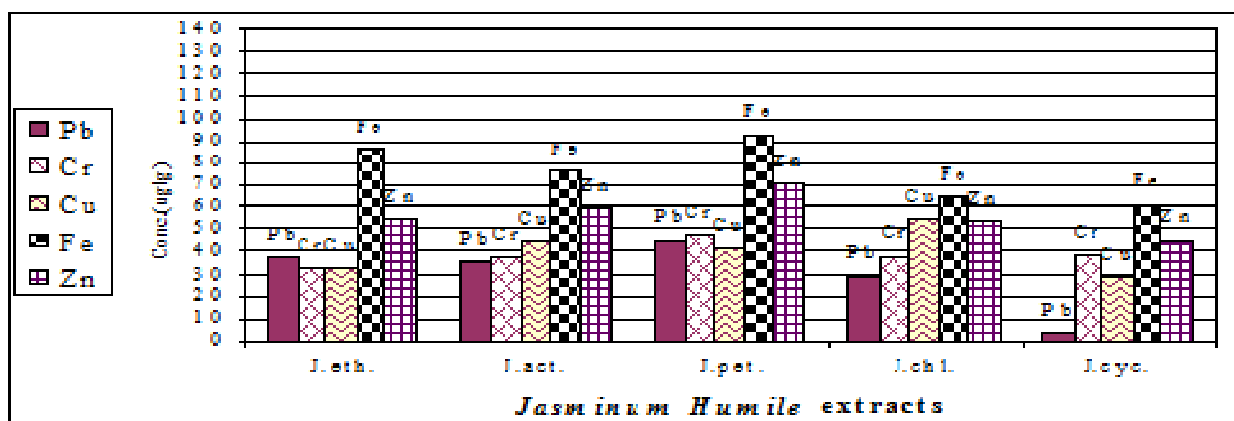


Fig. (8): Conc. of metals in *Jasminum Humile* extracts

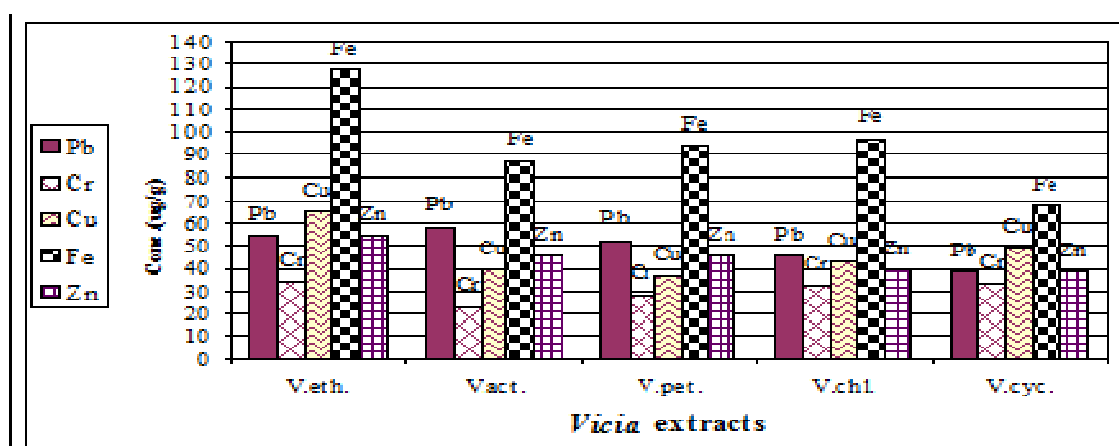


Fig. (9) : Conc. of metals in *Vicia* extracts

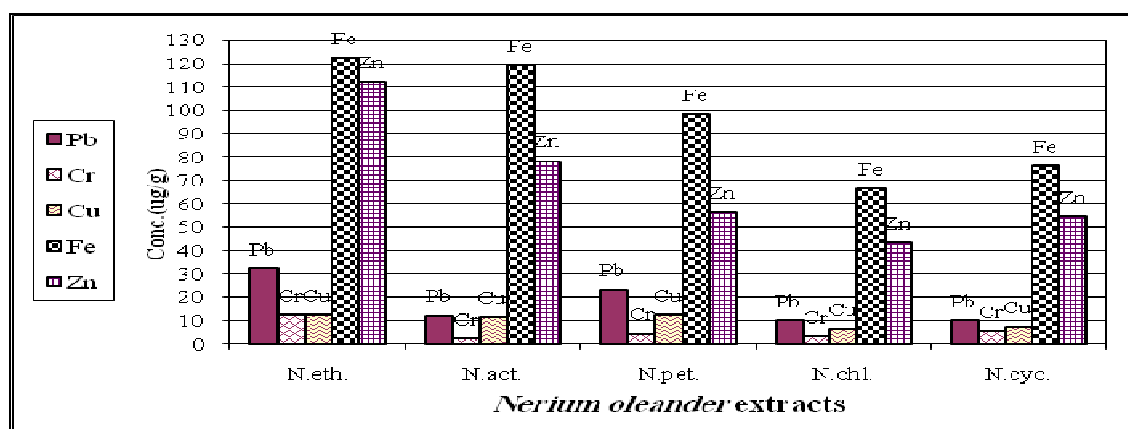


Fig. (10) :Conc. of metals in *Nerium oleander* extracts

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