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Characterization of Eucalyptus globulus found in Provinces of Pakistan and its applications related to control Environmental Pollution

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ABSTRACT
Eucalyptus consist of 500 species growing in all over the world including Pakistan, native to Australia. The reason of selection of globules specie is that it has a great medicinal importance, being used as anti diabetic, anti fungal, anti bacterial, antiviral, analgesic and to relief wound. The function of pH, moisture content and metal ions in biological system is so valued that their imbalance can cause severity. The focus of the present study was to estimate the pH, moisture content and metal ions in *Eucalyptus globules*. To know the concentration of toxic metal ions is very significant as it becomes hazardous if enter in biological system and exceed the acceptable limit. Metal ions estimated at Atomic absorption spectrometer by using the accurate standard and sample preparation method. It is experimentally confirmed that moisture content and concentration of Pb, Cu Ni, Cd & Fe is dissimilar in polluted and unpolluted areas of Pakistan.

Keywords*: Eucalyptus globulus*, toxic metal ions, environmental conditions, contamination, health effects

1. INTRODUCTION
Eucalyptus globules is a medicinal plant [Fig-1] which is especially used for the lowering of water level in different areas of Pakistan and all over the world. There are different species which are found in Peshawer such as *Eucalyptus diversicolor Eucalyptus dundasi Eucalyptus* gomphocephala *Eucalyptus* slaubris *Eucalyptus* salmonophloia *Eucalyptus* brockwayi. It has been reported that plants are more sensitive to pollution than animals or man. Injury to vegetation caused by heavy metals has been well documented as a result of many botanical and chemical investigations during the past many years [1]Roadside soils often contain high concentrations of metallic contamination. The bioavailability and environmental mobility of the metals are dependent upon the form in which the metal is associated with the soil. Lead street dust and roadside soil has been extensively studied, and found to be present at elevated levels[2-5].Heavy metals are elements having atomic weight between 63.545 and 200.5g and a specific gravity greater than four[6]. Metals, such As Pb, Cu, Fe, Zn, and Cd are essential components of many alloys, wires, tires and many industrial processes, and could be released into the roadside soil and plants as a result of mechanical abrasion and normal wear. Analyses of roadside soil [7-11] and plants [12-14] revealed that they contain elevated levels of these heavy metals. The toxicity of these metals has also been demonstrated throughout history: Greek and Roman physicians diagnosed symptoms of acute lead poisoning long before toxicology became a science. Exposure to heavy metals has been linked with developmental retardation, various cancers, kidney damage and even death [15-17].In Pakistan, the major sources of heavy metals pollution are industrial effluents discharged from various processing industries. This increases the influx of metals, which can be transported by wind and water and thus become available to plants and animals.The total average annual rainfall is 6-8 inches (150-200 nm) [18]. The metal ions concentration either essential or non essential, critically affect the biological system of the human body[19].The aim of our study was to estimate the concentration of toxic metal ions, moisture and pH in polluted and unpolluted areas of Pakistan related to high traffic zones.

A

B

Fig-1: (A) Leaves of *Eucalyptus globulus* (B) Fruits of *Eucalyptus globulus*

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2. EXPERIMENTAL

2.1 *Materials and Methods*

2.1.1 *Sampling Sites*

The sampling sites were divided into four locations Peshawar, Khusdar, Karachi & Multan from KPK (Khyber Pukhtoonkhwa), Baluchistan, Sindh & Punjab provinces of Pakistan respectively. Samples of soil and plants from different locations shown in [Fig-2] collected by author from polluted areas.

2.2 *Sampling Procedure*

Samples were collected in dry season. The plant samples were collected and transported to the laboratory in polythene bags. The plants were washed with distilled and de-ionized water and the green shoots were dried at 90ºC for 24 hours. Sample filters were kept in polythene bags to minimize sample loss until the time of analysis.

2.3 *pH analysis in soil and bark & leaf*

Take 10g of air-dried soil (milled to <2mm) for soil pH. Add 25 ml of distilled water. Shake periodically for 15 minutes. Stir, insert electrode and measure pH. Powdered material of bark and leaf was suspended in 100 ml distilled water. After two hours filtered and clear solution was measured for pH.

2.4 *Determination of moisture content*

The fresh sample of leaf, soil and bark was selected for estimation of water content measurement .Initially weighed around 10 gm of each then dried to a constant mass in an oven at a temperature of 100°C.Final weight was recorded and % moisture calculated on the basis of fresh and dry masses in gm by using formula [19].

$$\% \text{ Moisture} = \frac{\text{Initial weight (gm)} - \text{Final weight (gm)}}{\text{Initial weight (gm)}} \times 100$$

Balochistan, City: Khusdar, Location: Khuzdar Engineering University
Sindh, City: Karachi, Location : Korangi Industrial Area near English Biscuit Factory
Punjab, City: Multan, Location: Gulgust colony
KPK (Khyber Pukhtoonkhwa), City: Peshawar, Location: Main G.T road
Reference Location (Controlled area): P.E.C.H.S Govt Girls college block-2 Karachi, Sindh. Pakistan

Fig-2 Sampling Location of *Eucalyptus globulus*

2.5 *Sample Preparation and Analysis*

Plants samples were gently ground using electrical grinder. About 3g of dried leaves was accurately weighed and treated with dilute aqua regia in 1 L deionized water.The mixture was heated in a beaker until all matter dissolve and then cooled. The digested plant samples were then re-dissolved in 10% HCl to avoid precipitate formation. Here we did introduce HCl but we had blanks prepared in the same medium. So the presence of HCl could be minimized. Extracts were filtered through Whatman filter paper No.40, and the volume was adjusted to 50 ml with 10% HCl in polythene volumetric flask. Reagent blank for soil and plant filter were also prepared in the same way as those of samples. Metals analysis was carried out with a Flame Atomic Absorption Spectrometer (PERKIN ELMER- 2380).

3. RESULT AND DISCUSSION

Observed and reference pH values from bark of *Eucalyptus globulus* were reported in the [Table-1] are relatively acidic regardless location as all below from neutral point 5.3, 4.35, 5.33, 6.15 < 7.0 but soil pH is almost neutral such as 7.71, 6.86, 7.9 & 7.9 in sample from Balochistan, Sindh, Punjab and KPK respectively. pH in sample of leaf of *Eucalyptus globulus*from polluted location of Balochistan and Sindh is acidic (5.09, 4.17 < 7.0) but from Punjab and KPK is somewhat basic (7.59, 7.59>7.0) respectively .The pH of bark of *Eucalyptus globulus* in controlled reference sample from unpolluted location is also in acidic range 6.65<7.0 [Table-1] Concentration of Cadmium in (µg/g) is least in bark, soil and leaf in the range of (Cd:014~1.56 µg/g) as compare to other toxic metal ions, such as (Pb :34.54~79.72 µg/g), (Cu :6.51~47.01 µg/g) (Ni :8.45~48.78 µg/g), and (Fe: 1.86~5.31 µg/g).The concentration of these metal ions in reference sample from un polluted location of P.E.C.H.S Karachi is (Pb:30.22~39.32 µg/g), (Cu: 5.95~8.99 µg/g) (Ni7.99~10.02 µg/g), (Cd:0.10~0.41 µg/g) & (Fe:1.11~1.91 µg/g) [Table-2] .The concentration of copper is lower in *Eucalyptus globulus* in unpolluted location of as compare to other polluted provincial locations such as, in reference bark 7.01 µg/g < Baluchistan bark µg/g 16.97~Sindh bark 10.88 µg/g ~Punjab bark 10.14 µg/g ~KPK bark 7.41 µg/g. The highest concentration of Pb was found in leaves of *Eucalyptus globulus* collected from Sindh and Punjab as compare Balochistan and KPK (Sindh: 79.72 µg/g, Punjab: 78.56 µg/g, Balochistan: 49.33 µg/g, KPK:42.95 µg/g). The air born lead is usually present in the form of insoluble particulate matter and bulk of it is retained in the soil and sediments with very little present in water in the soluble form. In Pakistan lead additives are still used as anti-knock agents. Least concentration of Copper was found in the soil collected from KPK as compare to Baluchistan, Sindh and Punjab 6.51 µg/g < 15.97 µg/g, 10.88 µg/g, 10.24 µg/g respectively. The soil of Pakistan partly residual, calcareous, derived from limestone, sandstone and dolomite, which are the dominant rock formations. Copper mainly comes from automobile radiator, component of engine, thrust bearings and bearing metals. Cu levels are found highest in soil samples of Balochistan (15.97 µg/g) Nickel in leaves and bark of *Eucalyptus globulus* collected from Balochistan is found highest (leaves: 40.08 µg/g, bark 45.45 µg/g) as compare to KPK (leaves: 10.72 µg/g and 9.45 µg/g) respectively. Ni also comes from oil combustion, Ni alloy pipes and wear and tear of cars. The concentration of Fe in bark in all samples of *Eucalyptus globulus* is almost in the same range as 4.40 µg/g from sindh, 4.35 µg/g from Punjab, 4.30 µg/g from KPK but least in Balochistan as 1.96 µg/g. 4.40 µg/g ~4.35 µg/g ~4.30 µg/g >1.96 µg/g [Table-2].Fe is easily leached form sulphide ores via the oxidative formation of soluble iron(II) sulphate and it is also removed quite readily form the silicate minerals. Once in solution Fe^{+2} salts are easily oxidized to Fe^{+3} and under even the mildest alkaline conditions the iron in this state is re – precipitated as the oxide.

Table-1: pH of bark, soil and leaf of Eucalyptus collected from four polluted locations of Pakistan

Samples	Ref (P.E.C.H.S)	Balochistan	Sindh	Punjab	KPK
Bark	6.65	5.3	4.35	5.33	6.15
Soil	7.90	7.71	6.86	7.9	7.9
Leaf	7.59	5.09	4.17	7.59	7.59

Table-2: Metal ions concentration in bark, soil and leaf of *Eucalyptus globulus* collected from four locations of Pakistan

Sample	Location	Pb (µg/g)	Cu (µg/g)	Ni (µg/g)	Cd (µg/g)	Fe (µg/g)
Bark	Balochistan	35.56	16.97	45.45	1.48	1.96
Soil	-	34.54	15.97	44.45	1.11	1.86
Leaves	-	49.33	18.83	40.08	1.56	2.87
Bark	Sindh	49.56	10.88	37.89	1.33	4.40
Soil	-	48.52	10.88	38.39	1.34	4.97
Leaves	-	79.72	47.01	48.78	1.41	4.128
Bark	Punjab	45.52	10.14	36.53	1.24	4.35
Soil	-	46.45	10.24	37.52	1.32	4.85
Leaves	-	78.56	46.54	45.56	1.40	3.92
Bark	KPK	40.87	7.41	9.45	0.55	4.30
Soil	-	40.87	6.51	8.45	0.14	5.31
Leaves	-	42.95	9.26	10.72	0.37	4.68
Bark	Ref (P.E.C.H.S)	33.26	7.01	8.95	0.41	1.11
Soil	-	30.22	5.95	7.99	0.10	1.41
Leaves	-	39.32	8.99	10.02	0.12	1.91

The percentage of moisture [Table-3-7] is almost same in leaf and bark of *Eucalyptus globulus* collected from all location as 4.85 %in leaf and 4.95% in bark from Balochistan, 98.1% in leaf and 96.3% in bark from Sindh, 32.35% in leaf and 33.65% in bark from Punjab, & 23.91% in leaf and 20.22% in bark from KPK .The acidic nature of bark of *Eucalyptus globulus* showed that its medicinal use should be precautionary. The exceed concentration of Cu accumulation in biological system is a main cause of Wilson disease and Cu in *Eucalyptus globulus* is high from all

polluted areas. Traditional use of water extract of leaves of *Eucalyptus globulus* should be avoided as diverse range of toxic metal ions was observed experimentally in different areas of Pakistan affected by environmental pollution. There are no guidelines to establish a permissible level of metals in herbs. By monitoring the level of metals in medicinal plants one can be able to indicate the level of environmental pollution in that area. Environmental pollution effects badly on plant growth and its genotype. The sources of environmental pollution are varied, Industries, metallurgical process, traffic fumes and also advanced life style play a vital role in this regards, which may cause serious health hazards such as renal failure, skin diseases, and liver damage[20].

Table-3: Moisture content of bark, soil and leaf – *Eucalyptus globulus* collected from Balochistan

Eucalyptus globulus	Initial weight (gm)	Final weight (gm)	Difference	% Moisture
Bark	10.3 +/-0.01	9.8+/-0.01	0.5+/-0.01	4.85
Soil	10.1+/-0.01	10.0+/-0.01	0.1+/-0.01	0.99
Leaf	10.1+/-0.01	9.6+/-0.01	0.5+/-0.01	4.95

Table-4: Moisture content of bark, soil and leaf – *Eucalyptus globulus* collected from Sindh

Eucalyptus globulus	Initial weight (gm)	Final weight (gm)	Difference	% Moisture
Bark	10.0+/-0.01	0.37+/-0.01	9.63+/-0.01	96.3
Soil	10.01+/-0.01	0.44+/-0.01	9.57+/-0.01	95.6
Leaf	10.0+/-0.01	0.19+/-0.01	9.81+/-0.01	98.1

Table-5: Moisture content of bark, soil and leaf – *Eucalyptus globulus* collected from Punjab

Eucalyptus globulus	Initial weight (gm)	Final weight (gm)	Difference	% Moisture
Bark	10.4+/-0.01	6.9+/-0.01	3.5+/-0.01	33.65
Soil	10.4+/-0.01	9.8+/-0.01	0.6+/-0.01	05.76
Leaf	10.2+/-0.01	6.9+/-0.01	3.3+/-0.01	32.35

Table-6: Moisture content of bark, soil and leaf – *Eucalyptus globulus* collected from KPK

Eucalyptus globulus	Initial weight (gm)	Final weight (gm)	Difference	% Moisture
Bark	10.78+/-0.01	8.6+/-0.01	2.18+/-0.01	20.22
Soil	10.81+/-0.01	9.0+/-0.01	1.81+/-0.01	16.74
Leaf	10.12+/-0.01	7.7+/-0.01	2.42+/-0.01	23.91

Table-7: Reference controlled unpolluted location, P.E.C.H.S Govt Girls college block-2 Karachi, Sindh. Pakistan **%** Moisture content of bark, soil and leaf – *Eucalyptus globulus*

Eucalyptus globulus	Initial weight (gm)	Final weight (gm)	Difference	% Moisture
Bark	1.7 +/- 0.01	0.8+/- 0.01	0.9+/- 0.01	53
Soil	53.8+/- 0.01	53.5+/- 0.01	0.3+/- 0.01	56
Leaf	37.4+/- 0.01	23.2+/- 0.01	14.2+/- 0.01	38

4. CONCLUSION

The present study illustrate that the barks of *Eucalyptus globulus* are of acidic nature and its indigenous use as water extract should be avoided as biological system rely on basic pH .Location wise concentration of Pd, Cu, Ni, Cd & Fe is different in soil, leaf and bark samples. It means that *Eucalyptus globulus* is best indicator for weather and environmental verifications such as least concentration values of copper were found in bark which were collected from controlled reference unpolluted area of Karachi, Pakistan.

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