

Influence of Cd on Physiological Disturbances, Essential Oil Content and Composition on Palmarosa (*Cymbopogon Martinii*)



Biology

KEYWORDS :

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ABSTRACT

The supposition assumed in the present investigation was that some essential oil bearing crops could be grown as alternate to conventional crops in heavy metal enriched soils. Investigations were carried out to evaluate the dose dependent effect of Cd on physiological changes and on essential oil content and composition. To sand culture grown Palmarosa plants different doses of Cd (ppm) consisting of 1- 0.0, 2- 1.25, 3-2.5, 4- 5.0, 5- 10.0, 6- 15.0, 7-20.0 were supplied. With increasing dose and duration plant growth characters as plant height, fresh weight and dry weight of shoot & root decrease as a result total plant biomass also decreases. Chlorophyll content and carbon-di-oxide exchange rate decrease indicating decrease in growth of plant. Oil yield was highest at nil Cd supply. % geraniol was highest at 2.50 ppm while geranyl acetate was highest at nil Cd supply; indicating effect of Cd on biosynthetic pathway. Cd (mg/Kg dry wt.) content was higher in roots than in shoots. The results support that Palmarosa can be grown up to 20 ppm Cd concentration.

1. Introduction

Heavy metal contamination of cultivable soils is now recognized as a major problem affecting growth and productivity of cereals and other edible crops and their products as well. Decontamination of heavy metals by physical means (excavation, digging) or chemical means (leaching) is very expensive (Salt et al. 1995). Hyper accumulator plants that have the ability to phytoextract metals from soils show potential applicability for removing heavy metals from soil such as Mustard, Astragalus. Few recent studies have suggested that aromatic plants also have the capability to accumulate heavy metals (Zheljazkov et al. 2006 and other references cited there in). Cd is one of the most prevalent heavy metal present on agriculture soils. Crops such as rice, wheat, corn and soybean accumulate varying concentration of Cd in shoots, roots and seeds thereby affecting quality of grain. (Sela, M. et al. 1988). In this study the dose dependent of heavy metal Cd on the growth essential production and photosynthetic disturbance of commercially important essential plant palmarosa (*Cymbopogon martini*) was investigated to determine the suitability of the plant to grow in Cd rich soil using standardized sand culture technique. Palmarosa is an important aromatic cash crop grown widely for its essential oil. The essential oil is as an aromatic agent in the food, pharmaceutical, perfumery, cosmetic and aromatherapy (Dubey et. al. 2000).

2. Materials and methods

2.1 Plant material

The slips of Palmarosa (*Cymbopogon martini* cv. PR1) were obtained from the farm nursery of Central Institute of Medicinal & Aromatic Plants, Lucknow. Uniform slips of palmarosa were planted in 5l plastic pots filled with acid washed clean silica sand to remove impurities (Agarwala, S. C. and Sharma. P, 1961. Srivastava and Luthra, 1991). Balanced nutrient solution of Hoagland and Arnon, 1938 (except Fe which as FeEDTA) (Hewitt, E. J. 1966) was supplied to the plants and these served as control. Three pots with one plant per pot were maintained for each treatment. Plants were maintained in glasshouse at ambient temperature of 30-35°C and average light intensity 800-1000 μ E/m²/s. After establishment of slips (3 weeks) plants were supplied with different doses of Cd (CdNO₃) which consisted of (ppm) 1-0.0, 2-1.25, 3-2.50, 4-5.0, 5-10.0, 6-15.0, and 7-20.0, supplied along with Hoagland solution. 200 ml of the solution were supplied to the respective treatments. On the 7th day the pots were flushed with distilled water. Three replicates were maintained for each treatment. Plants were harvested after 16 weeks of treatment.

2.2 Harvesting

After the treatments, the plants were carefully uprooted from sand and separated into shoots (leaf-stem) and roots. The fresh weights were recorded. A known weight of fresh shoots was retained for extraction of essential oil and determination of Cd.

The remaining fresh shoot and root were dried in oven to a constant weight.

2.3 Photosynthetic efficiency measurement

Carbon-di-oxide rate (CER), Transpiration rate (TR) and Stomatal conductance (SC) - of third leaf of each treatment was measured using a portable photosynthesis system model CI-310 PPS (C.I.D Instruments U.S.A.) (Srivastava and Srivastava, 2007).

2.4 Chlorophyll content

A known weight of third leaf tissue of each treatment was extracted with 80% acetone and extract made up to a known volume. The absorbance recorded on a Helios spectrometer (Thermoelectron Corporation, U.K.) and chlorophyll content calculated according to method of Arnon (1949).

2.5 Extraction of essential oil

For determining total essential oil in shoot, a known weight of shoot was subjected to steam distillation in a Clevenger's apparatus (Clevenger, 1928) and the constituents analyzed by Gas chromatography (Srivastava et al, 2003). The GC analysis was performed using a Perkin Elmer (model 3920 B) apparatus equipped with FID (flame ionization detector) and a stainless steel column (2 m \times 3 mm i.d.) packed with 10% FFAP (free fatty acid phase) on chromosorb WAW (80-100 mesh). The operating conditions were: column temperature isothermal at 165°C, injector and detector temperatures 200 and 250°C, respectively. Nitrogen and hydrogen flow rates were adjusted to 30 and 28 ml/min., respectively. The geraniol and geranyl acetate peaks were identified by co injecting authentic standards and quantified using a Varian integrator (model 4400).

2.6 Determination of Cd content in shoot and root

Dried shoot and root were powdered in a Wiley mill and digested in acid. The Cd content was determined by a Perkin Elmer Inductively Coupled Plasma Spectrometer Model Optima 5300V (Srivastava et al. 2003).

2.7 Statistical analysis

The results presented are the mean values of three replicates and statistically analyzed for significance by analysis of variance (Singh and Choudhry, 1979).

3 Results and discussion

Plants were harvested when the symptoms were at their peak. These symptoms included shortening of the internodes hence decrease in plant height, curling of the leaves and leaf yellowing (Figure 3). The parameters indicating plant growth as plant height shoot & root fresh and dry weight decrease with increasing dose and duration of Cd treatment (Table 1). Similar results of decrease in plant growth characters have been reported in maize and in pea under cadmium deficiency (Lozano-Rodriguez et al. 1997). One of the most prominent effects of metal is on

the decrease in photosynthetic rate consequently reducing biomass. In the present study the parameters determining leaf gas exchange as CO₂ fixation, stomatal conductance, transpiration rate, and chlorophyll content decrease consequently resulting in decline of plant biomass. In many aromatic and medicinal plants mineral metals (including heavy metals as Copper) play important role in plant growth. Supplies of B at 2.5 ppm and Fe at 22.4 ppm significantly increased herb yield in *Artemisia annua* (Srivastava and Sharma 1990). Opium poppy (*Papaver somniferum*) is highly sensitive to deficiency of B supply (<0.05 ppm) beyond that level significant increase in biomass was observed (Srivastava et al. 1985). In *Cineraria maritima* higher doses of B at 1.0 mg/Land Zn at 0.1 mg/L were beneficial for biomass production (Srivastava and Bagchi 2006). These experiments show that different aromatic plants have different requirement of micro metal for optimum biomass yield.

Table-1 Effect of various Cd treatments on growth parameters of Palmarosa.

Treatment of Cd (ppm)	Plant height (cm)	Shoot fresh weight(g/plant)	Shoot dry weight(g/plant)	Root fresh weight(g/plant)	Root dry weight(g/plant)	Fresh plant weight (g/plant)
0.0	69.3	93.73	29.08	58.46	10.25	152.22
1.25	62.0	91.38	25.70	46.98	8.19	138.47
2.50	60.3	86.20	25.52	28.52	6.14	115.72
5.0	57.3	83.55	22.27	27.49	5.31	109.18
10.0	53.3	63.50	21.23	23.57	4.47	87.08
15.0	53.3	60.95	12.47	23.56	4.44	82.25
20.0	45.3	50.79	10.88	21.89	4.12	75.68
SEM	1.90	1.19	0.79	7.43	0.13	1.42
SED	2.69	1.69	1.12	10.51	0.18	2.02
CD 5%	5.75	3.63	2.41	22.0	0.39	4.32
CD1%	8.01	5.05	3.36	31.3	0.54	6.02

Cd is not an essential micro metal for the plants, it is neither a part of any enzyme nor involved in any metabolic system. Under conditions of excess Cd conditions plant avoids Cd accumulation in the cytosol by compartmentalization of Cd in vacuoles, deposition in cell walls, and bind to organic molecules as phytochelatin or high molecular weight protein (Lozano-Rodriguez et al. 1997). In pea and maize plants total Cd concentration in shoot and root tissues was similar in both plants (Lozano-Rodriguez et al. 1997). In the present study with increase in Cd dose the content in shoot and in roots increase, however the overall content in roots was higher than in shoots (Figure 1 & 2).

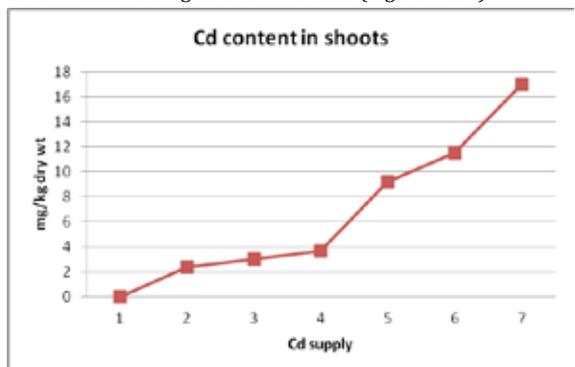


Figure-1 - Cd content in shoot of Palma rosa as Cd supply (ppm). 1- 0.0, 2-1.25, 15.0, 3-2.50, 4-5.0, 5- 10.0, 6- 15.0, 7-20.0.

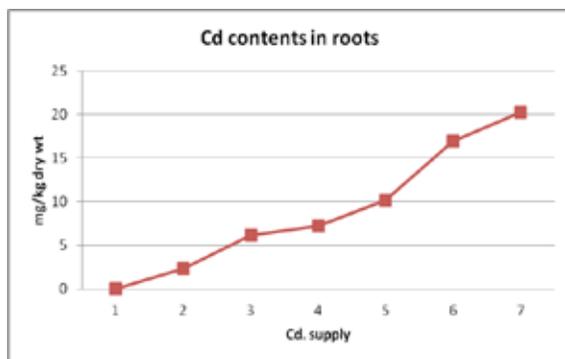


Figure-2 Cd content in root of Palmarosa as a function of Cd (supply) (ppm) 1- 0.0, 2-1.25, 3-2.50, 4-5.0, 5-10.0, 6-15.0, 7-20.

The most significant metabolic effect was observed on the photosynthetic efficiency of plants. The photosynthetic pigment chlorophyll was highest at nil Cd supply and it continually decreased with increase in Cd supply. Carbon-di-oxide exchange rate, transpiration rate and the stomatal conductance of the leaves were highest at nil Cd supply (Table 2). A higher supply inhibited photosynthetic efficiency and hence the overall growth of plants. In pea and maize also the root and shoot fresh weight also decreased concomitantly to Cd supply (Lozano-Rodriguez et al. 1997). Decrease in plant height and dry weight decreased with increasing Cd supply in essential oil crops as Mint, Basil and Dill (Zheljakov et al 2006).

Table 2- Effect of various Cd treatments on photosynthetic pigment and photosynthetic rate of Palmarosa.

Treatment ppm Cd	Total chlorophyll (mg chl/g leaf fresh wt.)	CO ₂ exchange rate (μmole/m ² /s)	Transpiration rate (mol/m ² /s)	Stomatal conductance (mol/m ² /s)
0.0	2.89	3.9	0.41	0.45
1.25	2.74	3.46	0.26	0.39
2.50	2.38	2.66	0.37	0.40
5.0	1.7	2.26	0.23	0.30
10.0	1.35	1.8	0.21	0.34
15.0	1.11	1.7	0.19	0.28
20.0	0.91	1.56	0.22	0.19
SEM	0.14	0.27	0.03	0.01
SED	0.20	0.39	0.04	0.02
CD 5%	.043	0.83	0.10	0.05
CD1%	0.60	1.16	0.144	0.06

Effect of Cd treatment resulted in gradual decrease in total oil content with increasing dose from 90 to 25%. However major constituents of oil, geraniol shows decreasing content with increase in dose (82 to 90%) whereas geranyl acetate shows decreasing content from 10 to 3% (Table 3). The variations in composition of essential oil show the influence of Cd on the biosynthetic pathway of essential oil. The application of Cd to other essential oil bearing crops as Dill and Basil altered the essential oil composition. In Peppermint menthol content in the oil was reduced as a result of Cd application compared to control (Zheljakov et al 2006). These authors reported that no content of Cd in the oil of Peppermint, Basil & Dill were detected hence these crops could be safely grown as alternative crops in Cd enriched soils without significant alteration in oil (Zheljakov et al 2006).

Table-3 Effect of various Cd treatments on oil yield and content of Palmarosa

Treatment ppm Cd	Oil yield %	Geraniol %	Geranyl acetate %
0.0	90	82	10.66
1.25	41	87	6.66
2.50	32	90	5.66
5.0	33	89	6.33
10.0	29	88	6.66
15.0	27	90	3.6
20.0	25	89	3.0
SEM	0.02	0.83	0.43
SED	0.02	1.18	0.61
CD 5%	0.06	2.52	1.32
CD1%	0.08	3.52	1.83

Our results demonstrated that Cd is not removed from the tissues during the process of steam distillation. This finding strongly supports the use of aromatic plants particularly Palmarosa as an alternative crop for Cd enriched soils that can yield metal free product and at the same time remove Cd from the soil as shoot and roots are enriched in Cd content withdrawn from soil.



Figure-3. Comparative view of visual symptoms of Palmarosa. On the right (1) is control and on the left (7) is plants with a dose of 20.0 ppm.

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