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The impact of hedgerow to the lead content in plants cultivated adjacent to motorway

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ABSTRACT:

Lead pollution is a worldwide environmental challenge. The present study was intended to evaluate the lead content of the pot marigold *(Calendula officinalis* L.*)* plants collected from a test field adjacent to motorway. The test field was separated from the heavily trafficked (>10,000 vehicles/d) motorway by the blue spruce hedgerow which was located transversely in a distance of 12 m from roadside. The pot marigold inflorescences (commonly used as pharmaceutical herbs) and leaves were collected at distances of 20 to 80 m from the roadside; the lead content was estimated using the dithizone lead determination technique. The 2.5 m high dense spruce hedgerow reduced the lead contamination of pot marigold leaves for 40 m from the hedgerow; the average lead content of leaves was 12.5 µg pro 100 cm^2. Contrary, at the 60 m distance to the hedgerow, a peak in lead content (33 µg/100 cm^2) was found. The absolute lead contents for the inflorescences were much lower (2-8 µg/100 cm^2) and no unidirectional impact of the hedgerow on the lead content of inflorescences was found. In conclusion, the 2.5 m high dense spruce hedgerow was found to reduce the lead contamination of pot marigold leaves for a distance up to 40 m from the hedgerow. The lead content of pot marigold inflorescences was variable and it depends on additional environmental factors. Consequently, to reduce the health risks it is recommended to shield the pot marigold cultivation field from the traffic-originated lead pollution with an appropriate hedgerow.

Keywords:
Lead content, lead, pot marigold, *Calendula officinalis* L., environmental factors, dithizone lead determination.

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INTRODUCTION

The use of medicinal plants as remedies is an increasing trend both in developed and source limited countries (Hall and Nazir, 2005). The medicinal plants have been collected from their natural habitats for millennia, however due to the standardization and quality issues during the last 50 years the cultivated of medicinal plants have become an important source of raw material for naturally originated pharmaceutical formulations. The cultivation of pharmaceutical plants face many challenges, among them the pollution with heavy metals is one of the most important problems to be addressed (Ernst, 2004; Fu *et al.*, 2009; Jordan *et al.*, 2010). Though the usual administration of remedies of natural origin is timely limited, the potential heavy metal caused health risk is augmented due to two key factors. One hand, the natural medicines are concentrates of medical plants and secondly, the patients may be more susceptible to any damaging factor due to their illness. Worldwide, there are many alarming reports indicating that the collected medicinal plants, both from natural habitats as well as from medicinal plant fields contain heavy metal above limits set by the local authorities or international organizations (Caldas and Machado, 2004; Naithani and Kakkar, 2005; Obi *et al.*, 2006; Mishra *et al.*, 2007; Gasser *et al.*, 2009; Meena *et al.*, 2009; Baye and Hymete, 2010). From the other side, several medicinal plant species could act as the bio indicators for pollutants (Sarala Thambavani and Prathipa, 2012).

In our previous study it was unveiled that in a country with abolished use of lead containing fuel still traces of lead in medicinal plants can be detected, though the major contributor of the lead pollution was not the traffic intensity but the amount of precipitates in the test field area (Meos *et al.*, 2011).

The present study was aimed to evaluate the lead content in pot marigold (*Calendula officinalis* L.) inflorescences and leaves collected from a test field at various distances from a heavily trafficked motorway in Tartu, Estonia. The test field was separated from the motorway by a dense spruce 2.5-3 m high hedgerow. The shielding factor of a hedgerow is not studied so far in the context of the cultivation of the pharmaceutical plants.

The content of the carotenoids and flavonoids in pot marigold different cultivars growing in Estonia is well documented. Pot marigold crude drug and preparations are widely used both in Estonian folk and latest traditional medicine for internal (infusions, tinctures) as well external (ointments) use (Toom *et al.*, 2007; Raal *et al.*, 2009; Raal and Kirsipuu, 2011).

In the present study, the objective to choose the pot marigold plants was primarily the fact that the pot marigold plants are easy to cultivate and that the anthesis period is sufficient to collect the critical amount of inflorescences for lead content estimation. Thus, in the present study the pot marigold was used as the model plant of the cultivated medicinal plants in Estonia.

MATERIALS AND METHODS

Plant material

The plant material (pot marigold inflorescences and leaves) was collected in August to mid-September from a experimental garden of the University of Tartu located at heavily trafficked (>10,000 vehicles/d) motorway close to the administrative town border of the Tartu City, Estonia (coordinates are N:58.36194; E:26.67889). The test field was separated from the motorway by the dense, 2.5-3 m high blue spruce (*Picea pungens* Engelm. *f. glauca* (Regel) Beissn.) hedgerow which was located transversely in a distance of 12 m from roadside. The hedgerow contained also a few Wolf's lilac (*Syringa wolfii* C.K. Schneid.) bushes but these low and rare individual bushes were not considered to contribute much to the overall shielding effect of the hedgerow. The width of the hedgerow was around 2 m (at the 1 m from ground) and did not contain any gaps between the individual spruces. The test field reached from the hedgerow longitudinally for 200 m and transversely to 100 m. The pot marigold seeds of the present study were disseminated in spring with constant 10 m crosswise intervals starting 20 m from roadside (i.e., 8 m from the hedgerow) to 80 m. The seeds were obtained from pot marigold (unknown cultivar) plants cultivated and collected at the same field a year before.

The first samples of plant material were taken from a distance 20 m off the motorway, and then with each 10 m intervals farther from the motorway, the samples were collected weekly during seven consecutive weeks always from the same spot. The leaf and inflorescence samples were taken from each collection spot.

The surface area of the plant material was calculated using raw leaves and inflorescences which were put onto a standard paper sheet of white A4 printer paper (5.154 g, 623.7 cm^2), their contours were marked and cut off. The surface areas of plant material were calculated on the paper weight basis. The plant material was stored until the material was dried in paper bags left open; after drying at normal (23±2°C) temperature during 10 days the bags were air-tightly closed and kept at room temperature until analyses of lead content. As it was shown by (Glover-Amengor and Mensah, 2012), the beta-carotene level in plant material was less the content of some vitamins affected by drying at room temperature.

All chemicals used were obtained at the local Sigma-Aldrich or Fluka distributor and were at least of analytical grade of purity.

Estimation of lead content

The dry plant material was transferred into clean vials containing 20 ml of 0.1 N nitric acid heated to 70°C and shaken for 2 min, and then filtered. 3 ml of the liquid was taken and transferred to the vials in quadruplicate (Meos *et al.*, 2011), and finally the content of lead was measured in four parallels using the dithizone determination technique. The actual determination of lead content was carried out as described in detail by Fischer and Leopoldi in 1940 (Fischer and Leopoldi, 1940) with subsequent colorimetric estimation at 510 nm in reference to the standard solutions (0.25-2.5 mg/ml). All lead content data are expressed as μg/100 cm^2 ±SEM. The environmental factors were not specially recorded, but the weekly precipitates were estimated roughly using a glass cylinder for the collection of precipitates. Since the exact estimation of the amount of precipitates was out of the scope of the study and thus only supportive, the amount of weekly precipitates is given as "+" for <10 mm per week and, "++" for >10 mm per week.

RESULTS AND DISCUSSION

The major finding of this study was that the lead content of pot marigold leaves and inflorescences was

Table 1 Lead content in pot marigold (*Calendula officinalis* L.) leaves and inflorescences collected during the full size period and analyzed by weeks

Collection week	Lead content of leaves	Lead content of inflorescences	Precipitates
1	18.1±6.3	3.1±0.8	+
2	19.8±4.7	7.8±1.9	+
3	15.5±5.0	7.6±0.9	+
4	19.0±6.8	6.3±0.4	++
5	12.1±2.1	4.2±1.9	+
6	12.6±1.3	6.5±2.6	+
7	16.6±3.1	6.0±1.3	+

The lead content of leaves and inflorescences is given as (μg/100 cm^2±SEM). For the precipitates, "+" = <10 mm per week , "++" = >10 mm per week (rough estimates).

stable for the 30 m from the hedgerow with a subsequent peak in lead content of leaves at the distance of 60 m from roadside while the inflorescences did not exhibit such an increase of lead content (Figure 1). Further, this finding clearly shows that it is recommendable to shield the pot marigold collection filed from the motorway by a natural barrier such as the hedgerow. Nevertheless, one could have been expected that the lead traces in plants are declining coherently with the distance from the motorway, however such a clear result was not found. The lead contamination peak at 60 m distance from the roadside can be due to the termination of the shielding effect of the hedgerow and most likely at this distance the wind and precipitates contribute to the abrupt increase of the lead content. The latter idea is supported from our previous study (Meos *et al.*, 2011) where we demonstrated that the amount of precipitates is a critical factor of the magnitude of the lead contamination of pot marigold plants. On the other hand, the effect of precipitates is rather short and the precipitates do not cause long-lasting increase of the total lead content. When the lead content of the pot marigold leaves was analyzed by weeks during the period when the plants were full size, only moderate fluctuations in lead content were found (Table 1). Notably, in line with our previous study (Meos *et al.*, 2011), the maximal lead content of leaves was found in the collection week four when the amount of precipitates was higher, too.

Though numerous studies focused on lead contamination of plants from areas close to roads have been published also earlier, the actual factors for contributing the lead contamination of plants collected from vicinity of motorways are not well defined. In an extensive study from Pakistan, the lead content of wheat leaves gradually decreased as the collection spots were extended farther from the road vicinity (Lone *et al.*, 2006). (Caselles, 1998) reported that lead content of citrus fruits collected from roadside dropped when the collection spot was far from roadside. Further, the authors concluded that a considerable amount of lead pollution was on the surface of the citrus plants and a substantial amount of this contamination can be washed out. This study supports our idea that considerable part of the lead contamination of traffic origin is only superficial and thus may be depending on the environmental conditions. In another extensive study, (Shakour and Nasrallaa, 1986) reported that lead and cadmium contents of clover plants are highly dependent on traffic density and distance from the road while the authors also reported that at least part of the lead

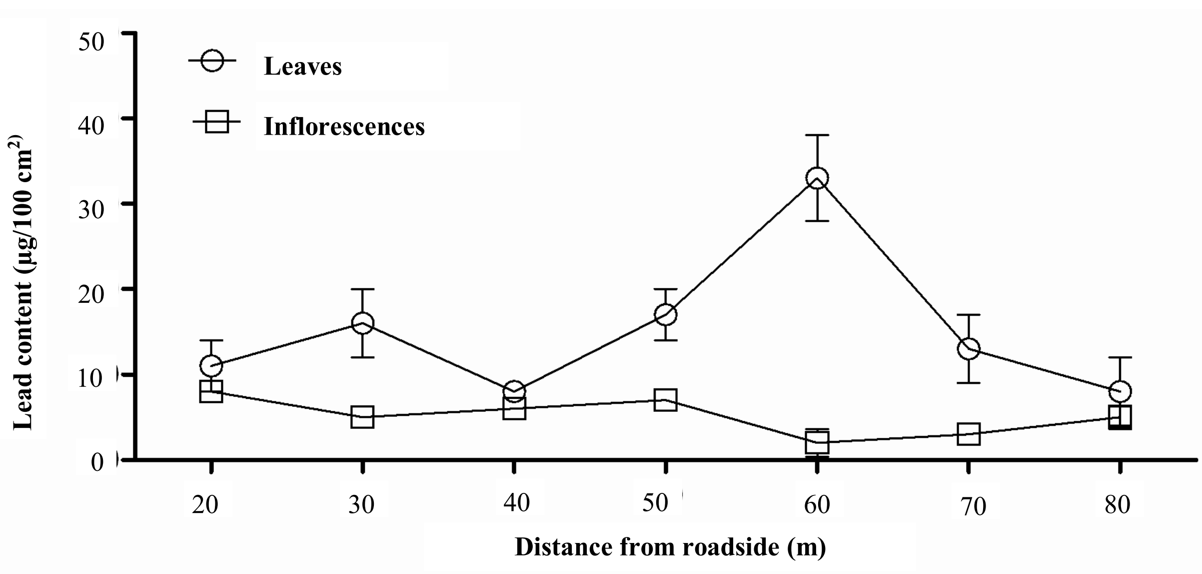

Figure 1 Lead content in pot marigold (*Calendula officinalis* L.) leaves and inflorescences.

contamination was removed by rinsing the plant material studied. Finally, in a study of (Bakirdere and Yaman, 2008), the lead content of roadside soil generally decreased with increasing distance from the motorway and there were big differences in the concentration of lead on two sides of road due to strength and direction of the wind and height of the buildings near the road. Altogether it shows that far more factors than simple distance from roadside has to be taken into account when interpreting the lead content data of roadside cultivated plants.

The role of the hedgerow as a shield is not studied in the context of trace element pollution of the medicinal plants. In a recent study it was found that the roadside trees exhibited high values of trace metal content while higher lead and iron values were found closer to roads and on the road-proximal rather than road-distal sides of trees. Further, the highest pollutant values occurred on tree leaves next to uphill rather than downhill road lanes (Maher *et al.*, 2008). Putting the latter result into the context of our present study, one could hypothesize that the roadside shielding eliminates at least a part of the traffic originated lead contamination and is therefore recommended to ensure the reduction of the medicinal plants lead contamination. On the other hand, as evidenced by the peak lead content found, it seems that the 2.5-3 m height of the spruce hedgerow is not high enough to eliminate completely the lead contamination, particularly as other environmental factors contribute the total lead contamination as well.

Furthermore, though the intensity of the traffic and the environmental factors such as the winds and precipitates are the key contributors of the roadside cultivated plants trace metal contamination, also some other interesting findings to be considered in the context of the present study have been already done. First, (Naveed *et al.,* 2010) found that in summer the heavy metal contamination of roadside plants is higher, though partly this finding is associated with more intense traffic in summer. Secondly, the roadside dust accumulation, directly associated with the lead contamination, is a plant specific phenomenon (Prusty *et al.*, 2005). The seasonal effect or the specific capacity of the pot marigold plants to accumulate the traffic originated lead is not known and remain to be elucidated in the further studies.

CONCLUSION

The 2.5-3 m high dense blue spruce hedgerow reduces the lead contamination of pot marigold leaves for a distance up to 40 m from the hedgerow. The lead content of pot marigold inflorescences is variable and it depends on additional environmental factors. Consequently, to reduce the health risks it is recommended to shield the pot marigold cultivation field from the traffic-originated lead pollution with an appropriate hedgerow.

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