**RESEARCH ARTICLE** 



# Tree species as a biomonitor of metal pollution in arid Mediterranean environments: case for arid southern Tunisia

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#### Abstract

We investigated the accumulation of Zn, Cu, Pb, and Cd in the soil and the leaves and bark of five common tree species (Eucalyptus occidentalis Endl., Acacia salicina Lindl., Cupressus sempervirens L., Casuarina equisetifolia L., and Tamarix aphylla (L.) Karst.) in the city of Gabès Tunisia to elucidate their bioaccumulation potential and determine their usefulness as biomonitors of metallic pollution in arid urban areas. Our results indicated that the bark had higher mean concentrations of Pb and Cd than leaves. In contrast, the leaves had higher mean concentrations of Zn and Cu than bark. No hyperaccumulation was detected for any of the analyzed metals in any of the studied species. E. occidentalis and T. aphylla had the highest mean concentrations of the investigated metals in leaves and bark. Based on the calculated metal accumulation index (MAI) values, these two species accumulated more metals than other studied tree species. Likewise, the concentrations of Zn, Cu, Pb, and Cd in soil had significant positive correlations with that in leaves and bark. Accordingly, E. occidentalis could be used for biomonitoring in arid areas subjected to industrial and traffic pollution. T. aphylla would be a good alternative when native species are a priority.

Keywords Metallic pollution · Bioaccumulation · Biomonitoring · Eucalyptus occidentalis Endl · Tamarix aphylla (L.) Karst · Urban arid environments

# Introduction

Air contamination by toxic elements is an environmental concern worldwide (Pulford and Watson 2003; Ugolini et al.

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2013; Janta and Chantara 2017; Jeddi and Chaieb 2018). In urban environments, metals are generated from human activities, such as vehicular traffic, fossil fuel emissions, and various industries (Al-Taani et al. 2019). Toxic levels of metals can rapidly deteriorate urban environments and threaten human livelihoods and development (El-Hasan et al. 2002; Abdallah et al. 2006; Al-Khashman et al. 2011; Farahat and Linderholm 2015). Trace metals can transfer into plant tissue and accumulate in the food chain, even in traceable amounts (Shahid et al. 2017).

Biomonitoring of air pollution using plants has received increasing attention in the last two decades as it is an efficient, easy-to-perform and inexpensive tool to follow contamination dispersion in the atmosphere (Pacheco et al. 2001; Senhou et al. 2002; Rai 2016; Alahabadi et al. 2017; Solgi et al. 2020). As reported in previous studies (Bargagli et al. 1997), plants as passive biomonitors offer a high density of sampling points that are effective for outlining maps of airborne metal contamination in urban environments. Aničić et al. (2011) noted that biomonitoring approaches are based on the response of organisms that act as bioaccumulators. Moreover, good biomonitors should show a correspondence to some instrumental monitoring data (Nourouzi et al. 2015).

In addition to epiphytic plants (Techato et al. 2014), trees are among the main plant types growing in countries with polluted urban areas (Sawidis et al. 2011). They are usually widely distributed and easier to identify than moss and lichens (Tomaševič et al. 2008). Extensive research has been conducted on the possibility of using trees as effective biomonitors of environmental pollution (Celik et al. 2005; Sawidis et al. 2011; Hu et al. 2014; Norouzi et al. 2015; Alahabadi et al. 2017). Trees can absorb and accumulate significant quantities of potentially toxic substances, mostly on their outer organs, such as foliage and bark (El-Hasan et al. 2002; Hu et al. 2014; Alahabadi et al. 2017; Jeddi and Chaieb 2019). Tree leaves and bark as biomonitors of toxic element pollution have great ecological importance (Sawidis et al. 2011). Celik et al. (2005) noted that elemental analysis of bark and leaves could be a useful and cost-effective way to protect vulnerable urban areas. Moreover, due to the excellent availability and long lifespan of trees, experimental replication is possible after several decades (Pulford and Watson 2003).

There are numerous examples of tree species that have been used to successfully monitor air pollution in urban environments, including Cupressus sempervirens L. (El-Hasan et al. 2002; Farahat and Linderholm 2015), Populus tomentosa Carr., Sophora japonica Linn. and Catalpa speciosa (Warder) Engelm. (Liu et al. 2007), Phoenix dactylifera L. (Al-Khashman et al. 2011), Quercus ilex L. (Ugolini et al. 2013), Platanus orientalis L. (Norouzi et al. 2015), and Cassia fistula L. (Janta and Chantara, 2017). These studies have indicated that the capacity to accumulate metals not only depends on the spatial distribution of the trees, time of exposure, and climate but also on the tree's specific features, such as leaf area and surface texture. On the other hand, metal accumulation can be the result of both atmospheric incorporation of metallic particulate matter on aboveground plant organs and root uptake of soluble metals (Vázquez et al. 2016; Solgi et al. 2020).

Situated in the southeast of Tunisia, Gabès is a typical urban city that suffers from high traffic density. Furthermore, there are several medium-sized phosphate fertilizer plants in the eastern part of the city that have a local effect on urban environmental pollution. The Gabès region is one of the most polluted areas in the Mediterranean Basin, according to a recent study by the World Bank (El Zrelli et al. 2015). In Gabès city, evergreen trees are typical urban plants grown in large areas, such as roadsides, in both industrial and suburban areas. To date, the bioaccumulation and biomonitoring of metal pollution using trees has received little attention in this area; the few investigations that have been conducted remain unpublished. With extensive anthropogenic activities, the potential of tree species to monitor atmospheric pollution in this urban environment needs to be evaluated.

For this purpose, five common tree species (Eucalyptus occidentalis Endl., Acacia salicina Lindl., Cupressus

sempervirens L., Casuarina equisetifolia L., and Tamarix aphylla (L.) Karst.) in Gabès city were selected. The main objectives of the present work were to (1) determine the concentrations of Zn, Cu, Pb, and Cd in the soil, and the leaves and bark of the selected tree species; (2) evaluate the trees' total metal accumulation capacities using the Metal Accumulation Index (MAI); and (3) support urban tree advocates to choose tree species appropriate for biomonitoring metallic pollution in arid urban areas.

## Materials and methods

#### Study area

Gabès is the second-largest city in southeast Tunisia ( $33^{\circ}$  52' 53" N and  $10^{\circ}$  5' 53" E; Fig. 1), located about 13 to 20 m above sea level. The city covers 7166 km<sup>2</sup> and has a total population of about 374,300 (52 people km<sup>-2</sup>). Like other areas in developing countries, Gabès faces many environmental difficulties caused by its vulnerable ecosystem, poor urban management, and rapid development. It has a significant industrial complex, which is a major source of environmental pollution. It also suffers from heavy traffic pollutants due to the passage of automobiles, of which only a few uses unleaded fuel. Traffic density ranges from 4000 to 33,000 vehicles per day. The investigated area has an arid climate with average annual rainfall of 167–176 mm and average annual temperature of 18.8–19.3 °C.

### Plant sampling and analysis

Sampling was carried out in late spring 2016 from trees located at five sampling sites (same meteorological, air, and soil characteristics; Alahabadi et al. 2017) in the southeast of Gabès city. Five evergreen common tree species were selected: three exotic species-Eucalyptus occidentalis Endl. (Broadleaf tree), Acacia salicina Lindl. (Broadleaf tree), and Casuarina equisetifolia L. (The foliage consists of slender, much-branched twigs, bearing minute scale-leaves in whorls)-and two Mediterranean native species-Tamarix aphylla (L.) Karst. and Cupressus sempervirens L. (Both tree species foliage consists of small leaves reduced to tiny scales that ensheath the wiry twigs). All sampled trees had been growing in the chosen sampling location for about 20 years and were in good condition (vigorous, healthy). When tree showed anomalous growth or deformities, the closest healthy tree of the same species was selected. Five trees for each species were sampled (one tree per site), and about 50 g of leaves (old leaves from the previous year's shoots) and 50 g of bark from each species were collected into plastic bags for transportation to the laboratory. The leaf samples were collected from each direction with respect to the tree trunk, 1.5 m





above the ground (depending on the plant structure). At the same height, bark samples were taken from the stem using a stainless-steel knife. In the laboratory, the samples were ovendried at 80 °C for 24 h (leaf samples were washed with deionized water), before being ground and homogenized through a 0.2 mm sieve. Next, aliquots of 0.5 g were ashed at about 550 °C in a muffle furnace for 3 h before being mixed with 10 ml of HNO<sub>3</sub> (2.8%) solution (Farahat and Linderholm 2015). The extract was filtered into 50-ml polyethylene volumetric flasks and then diluted to the 50 ml mark with ultra-pure distilled water. The solutions were analyzed using flame atomic absorption spectrophotometer (Avanta GBC spectrophotometer, Australia), using an air-acetylene flame. Concentrations were expressed in mg kg<sup>-1</sup> based on dry weights.

## Soil sampling and analysis

Four soil samples were collected under the canopy of each selected tree species (one from each side) from 0 to 20 cm depth and pooled to obtain one composite sample. After transfer to the laboratory, the 25 samples were air-dried at room temperature then sieved through a 2-mm nylon sieve. Soil pH and electrical conductivity (EC) were determined in a soil: water (1:2) suspension (saturated paw method, AFNOR 1987) using a pH meter (model PH 500, Tunisia) and a conductivity meter (model CON 500, Tunisia), respectively. For metal concentrations, a 10 ml HNO<sub>3</sub> (65%) solution was added to a 0.5 g soil sample. The sample was then warmed for about 3 h at 150 °C, before adding 2 ml of HClO<sub>4</sub> (70%); digestion was continued by evaporation to near dryness,

before adding 2 ml of HCl (37%) and heating to 150 °C for about 15 min (Celik et al. 2005). Lastly, the solution was diluted to 25 ml with double distilled water. Concentrations of Cu, Zn, Cd, and Pb in the extracts were measured with an atomic absorption spectrophotometer (Avanta, GBC spectrophotometer, Australia), using an air-acetylene flame and expressed in mg kg<sup>-1</sup> based on dry weights.

#### Data analysis

#### Metal accumulation index (MAI)

The MAI of each tree species was calculated to compare their ability to accumulate multiple metals (Liu et al. 2007; Monfared et al. 2013):

$$MAI = \left(\frac{1}{N}\right) \sum_{j=1}^{N} I_j \tag{1}$$

where N is the total number of metals and  $I_j$  is the subindex for variable *j*.  $I_j$  was obtained by dividing the mean concentration value of each metal by their standard deviation.

## **Statistical analysis**

All data were analyzed using SPSS (16.0) for Windows. Oneway analysis of variance (ANOVA) was performed to test for differences in metal accumulation in leaves and bark. When the ANOVA showed significant differences among tree species, we used Tukey's HSD test to perform pairwise comparisons. Differences at the P<0.05 level were considered

Tree species	Zn	Cu	Pb	Cd	pН	EC
Eucalyptus occidentalis Endl.	156.8 ± 12.5	42.8 ± 7.2	$26.8 \pm 2.3$	$5.8 \pm 0.1$	$7.2 \pm 1.2$	$6.2 \pm 0.8$
Tamarix aphylla (L.) Karst.	$158.2 \pm 16$	$45\pm3.6$	$27.7 \pm 1.62$	$6.04\pm0.28$	$7.1 \pm 0.8$	$6.3 \pm 1.7$
Casuarina equisetifolia L.	$160.1 \pm 13.5$	$45.8\pm8.1$	$28.5 \pm 2.7$	$6.28\pm0.75$	$6.9\pm0.87$	$6.22 \pm 1.6$
Acacia salicina Lindl.	$157.6 \pm 8.5$	$44.6\pm4.6$	$26.2 \pm 4.6$	$5.54\pm0.47$	$7.4 \pm 1.05$	$6.1 \pm 1.58$
Cupressus sempervirens L.	$157.9 \pm 9.3$	$43.7\pm3.3$	$26.5 \pm 2.2$	$5.68\pm0.94$	$7.2\pm0.91$	$6.1 \pm 1.2$
<i>P</i> value	NS					

 Table 1
 Mean metal concentrations  $\pm$  s.d. (mg kg<sup>-1</sup>), pH, and electrical conductivity (EC, mS cm<sup>-1</sup>) in soils underneath the studied tree species

ANOVA, n = 5, NS: not significant

statistically significant. The simple linear correlation coefficient was calculated to assess the relationship between the estimated metals in soil and those in tree leaves and bark.

# **Results and discussion**

### **Soil properties**

Concentrations of metals measured in the surface soil beneath all tree species are given in Table 1. The total content of metals varied as follows: Zn > Cu > Pb > Cd. According to Kabata-Pendias (2010), normal Zn, Cu, Pb, and Cd concentrations in soil range from 70 to 400 ppm, 60–125 ppm, 100–400 ppm, and 3–8 ppm, respectively. Comparing the average values (ppm) obtained in this study—158.1 for Zn, 44.4 for Cu, 27.1 for Pb, and 5.9 for Cd—with average world values indicates that Gabès city soils are enriched in Zn and Cd, while Cu and Pb are lower than the world average.

Soils collected beneath *C. equisetifolia* and *T. aphylla* had higher metallic contents than the other tree species; however, no significant differences were found (P>0.05). The pH values ranged from 6.89 to 7.4, revealing neutral to slightly alkaline soils. Despite no significant differences between the measured pH values (P>0.05), the soil beneath *C. equisetifolia* and *T. aphylla* had the lowest pH values, conversely to metallic concentrations. This suggests that the availability of metals under these tree species increases with decreasing soil pH (Kabata-Pendias, 2011). The EC measurements showed a relatively equal distribution underneath different tree species. The high values of soil salinity can be attributed to the high rate of dry deposition in the country's climate, which is classified as an arid area (Al-Khlaifat and Al-Khashman, 2007).

#### Leaf and bark metal concentrations

The concentrations of Zn, Cu, Pb, and Cd (mg kg<sup>-1</sup> DW) in the leaf and bark samples are shown in Tables 2 and 3, respectively, and varied within a tree species. Bark had significantly higher Pb and Cd mean concentrations than leaves (P < 0.001), being 2.5-fold and 1.7-fold higher, respectively. This may be because Pb and Cd are not essential elements (Kabata-Pendias and Pendias, 1992). They interact with Fe and Mn oxides and Zn and Cu, which can reduce their uptake from the soil (Witte et al. 2004; Kabata-Pendias and Pendias, 1992). It has been reported that Pb uptake into a leaf from the soil is unlikely since Pb is an element with low plant mobility (Norouzi et al. 2015). Based on these findings, the high Pb and Cd contents obtained in bark samples come from atmospheric deposition, which agrees with the findings reported by Solgi et al. (2020), Kuang et al. (2007), and Ferretti et al. (1995). Kuang et al. (2007) noted that elemental concentrations in tree bark originate predominantly from the dry and wet deposition of atmospheric metals as well as phloem. Solgi et al. (2020) and Ferretti et al. (1995)

Table 2 Mean metal concentrations  $\pm$  s.d. (mg kg<sup>-1</sup>) in the leaves of the studied tree species

Tree species	Zn	Cu	Pb	Cd
Eucalyptus occidentalis Endl.	99.2 ± 5.2a	38.3 ± 3.2a	15.2 ± 1.1a	3.5 ± 0.06a
Tamarix aphylla (L.) Karst.	$94.7 \pm 6.5a$	47.8±1.6b	$13.4 \pm 1.4 b$	$2.1\pm0.07b$
Casuarina equisetifolia L.	$32.8 \pm 3.5b$	$12.6 \pm 1.8c$	$12.5\pm1.5b$	$1.3 \pm 0.23b$
Acacia salicina Lindl.	$24 \pm 2.5c$	$34.6\pm2.5d$	$11.3 \pm 1.03 bc$	$2.4\pm0.09b$
Cupressus sempervirens L.	$23.5 \pm 1.3c$	$10.3 \pm 3c$	$10.4 \pm 1.1c$	$1 \pm 0.09c$
<i>P</i> value	*	**	*	*

ANOVA, n = 5, levels of significance: \*P<0.05 and \*\*P<0.01

Different letters in the same row denote significant differences (Tukey's HSD test at P<0.05).

**Table 3** Mean metalconcentrations  $\pm$  s.d. (mg kg<sup>-1</sup>) inthe bark of the studied tree species

Free species	Zn	Cu	Pb	Cd
Eucalyptus occidentalis Endl.	44.5 ± 4.7a	$17.2 \pm 1.5a$	35.5 ± 5.3a	4.8 ± 0.8a
Tamarix aphylla (L.) Karst.	$41.5 \pm 5.1a$	$19.4\pm2.5a$	$37.2 \pm 3.8a$	$3.1\pm0.3a$
Casuarina equisetifolia L.	$26.8\pm2.2b$	$17.1 \pm 3a$	$29.1\pm5.2b$	$2.4\pm0.9b$
Acacia salicina Lindl.	$11.12\pm1.1c$	$13.4 \pm 1.2 b$	$28.3\pm3.6b$	$2.2\pm0.6b$
Cupressus sempervirens L.	$13 \pm 1.2c$	$14 \pm 2.4b$	$25.2\pm2.1b$	$1.5\pm0.4c$
<sup>D</sup> value	**	*	*	*

ANOVA, n = 5, levels of significance: \*P<0.05 and \*\*P<0.01

Different letters in the same row denote significant differences (Tukey's HSD test at P<0.05).

indicated that bark is a suitable biomonitor and indicator of local atmospheric deposition. Likewise, Sawidis et al. (2011) reported that any enrichment in trace metals in bark could be related to passive superficial adsorption. In contrast, we found that leaves had higher average concentrations of Zn and Cu than bark (1.9 and 1.8 times higher, respectively); this may be because Zn and Cu are minor trace elements that are crucial for all organisms and play an important role in biosynthesis (Serbula et al. 2012). However, the accumulation of metals by leaves is not strictly related to biological internal fluxes (Feng et al. 2011). Thus, high concentrations of Zn and Cu in the leaves of all studied tree species can be attributed to the high rate of dry deposition in the city's climate, which is classified as an arid area, and to higher foliar uptake (Shahid et al. 2017). On the other hand, the patterns of both metals in tree leaves corresponded with those obtained in soil samples, suggesting that both soil and leaves are exposed to the same metal sources (Solgi et al. 2020).

As shown in Tables 2 and 3, *E. occidentalis* and *T. aphylla* had the highest mean concentrations of Zn in leaves and bark, which did not differ significantly (P > 0.05). *T. aphylla* and *C. equisetifolia* had the highest mean concentrations of Cu in leaves and bark, respectively. *C. sempervirens* and *A. salicina* had the lowest mean concentrations of Zn and Cu in leaves

and bark. Typically, Zn and Cu concentrations are less than 150 and 30 mg kg<sup>-1</sup>, respectively (Kabata-Pendias and Pendias, 2001). In the present study, Zn concentrations were within the normal range, but E. occidentalis and T. aphylla leaves had higher Cu concentrations than the critical value. E. occidentalis and T. aphylla had the highest Pb concentrations in leaves and bark, respectively, while C. sempervirens had the lowest. Lead pollution is caused, on a large scale, by emissions from motor vehicles using leaded gasoline (Al-Khashman et al. 2011). Typical Pb concentrations in plants range from 5 to  $10 \text{ mg kg}^{-1}$ , with toxic concentrations from 30 to 300 mg kg<sup>-1</sup> (Kabata-Pendias and Pendias, 2001). None of the tree species in this study had leaf Pb concentrations outside the normal range. However, E. occidentalis and T. aphylla had toxic levels of Pb in bark. E. occidentalis had the highest mean concentrations of Cd in leaves and bark, while C. sempervirens had the lowest. In our study, all species had Cd concentrations far below the phytotoxic level of 5 mg kg<sup>-1</sup> (Kabata-Pendias and Pendias, 2001).

## Metal accumulation index (MAI)

To assess the overall performance of the trees in terms of metal accumulation, Liu et al. (2007) developed the Metal



Fig. 2 Mean values for the metal accumulation index (MAI) of leaf and bark in the five studied tree species

**Table 4** Linear correlation coefficients between soil metal concentrations in leaf and bark of the studied tree species

Tree species tissues	Soil metals				
		Zn	Cu	Pb	Cd
Eucalyptus occidentalis Endl.	Leaf	0.69*	0.79**	0.69*	0.61*
	Bark	0.7*	0.62*	0.85**	0.67*
Tamarix aphylla (L.) Karst.	Leaf	0.72*	0.88**	0.77*	0.56
	Bark	0.68*	0.74*	0.87**	0.42
Casuarina equisetifolia L.	Leaf	0.44	0.62	0.57	0.45
	Bark	0.21	0.48	0.3	0.11
Acacia salicina Lindl.	Leaf	0.13	0.38	-0.22	0.13
	Bark	0.42	0.12	-0.1	-0.25
Cupressus sempervirens L.	Leaf	-0.29	0.52	0.46	0.2
	Bark	0.18	0.26	0.50	0.47

\*P<0.05; \*\*P<0.01

Accumulation Index (MAI). According to the calculated MAI (Fig. 2), E. occidentalis had the highest value in leaves (7.36), and T. aphylla had the highest value in bark (8.17). These maximum MAI values were much lower than those reported by Liu et al. (2007) for Catalpa speciosa (Warder) Engelm. (53.8 in leaves), but higher than those reported by Hu et al. (2014) for Sabina chinensis (L.) Ant. and Juniperus formosana Hayata (3.89 and 3.32 in leaves, respectively). Foliar characteristics, such as mass, area, and surface morphology, can affect the potential of leaves to ensnare airborne pollutants (Sawidis et al. 2011; Rodriguez et al. 2012)-the larger and rougher the leaf's surface, the greater the accumulation of metals. Hu et al. (2014) and Alahabadi et al. (2017) suggested that trees that grow low to the ground or broadleaved trees should be used as fences between polluted and susceptible environments. Therefore, tree species such as E. occidentalis, with its large leaf surface area, and T. aphylla, with leaves more exposed to soil splash, could be used to bioaccumulate metals in these urban areas.

In the current study, *C. sempervirens* had lower MAI values than the other investigated tree species, which is consistent with data reported by Alahabadi et al. (2017) for *Pinus eldarica* Medw. and *Cupressus arizonica* G.. Sawidis et al. (2011) noted that leaves of Gymnosperm, with a thick impermeable cuticle that forms a smooth sheet over epidermal cells, are relatively resistant to dust retention.

## Leaf-bark-soil metal correlations

The correlation coefficients between metal concentrations in soil and the leaf and bark of tree species are shown in Table 4. Significant positive correlations (P<0.01 and P<0.05) were found between Zn, Cu, and Pb in soils and their respective

concentrations in leaves and bark of *E. occidentalis* and *T. aphylla*. Similarly, the Cd content in soil under *E. occidentalis* had significant positive correlations (P < 0.05) with that in the corresponding leaves and bark. This finding indicates that *E. occidentalis* and *T. aphylla* reflect the cumulative effects of environmental pollution and suggests their potential use for biomonitoring of these trace elements (Jeddi and Chaieb, 2018; Solgi et al. 2020).

## Conclusion

For the studied tree species, bark accumulated more Pb and Cd than leaves, while leaves had the highest concentration of Zn and Cu. E. occidentalis and T. aphylla had the highest mean concentrations of Zn, Cu, Pb, and Cd in leaves and bark. Moreover, according to the MAI values, E. occidentalis and T. aphylla had the greatest ability to accumulate metals, simultaneously. The results of this study indicate that E. occidentalis and T. aphylla are good bioaccumulators for the investigated trace elements. Their high accumulation capacity as well as significant positive correlations between Zn, Cu, Pb, and Cd levels in soil and leaves and bark indicates their potential use as a suitable vegetative cover for soil and air biomonitoring in polluted arid urban environments. Selecting tree species as an urban restoration tool is a crucial step for accumulating and monitoring metals from polluted arid environments. Their use must be evaluated carefully, considering their effect on hostile environments with a fragile ecosystem and other aspects of their ecology, particularly the risk of using alien species. Outside of its original distribution area, E. occidentalis may have disadvantages, such as low survival rates, poor growth, and unwanted changes in community composition and ecosystem function. However, its introduction is of interest for various reasons, including the production of fuelwood and furniture, and the metal bioaccumulation potential identified in our study. T. aphylla is a good alternative when native species are a priority.

Authors' contributions KJ and MF carried out experimental and analysis work. KJ and MC: design and interpretation of experiments. KJ and KH.M.S wrote the manuscript.

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**Availability of data and materials** Data and materials supporting the results of this study are available from the corresponding author upon request.

#### Declarations

**Ethical approval** The authors approve that the content of this manuscript has not been published in a refereed journal, and it is not being submitted for publication elsewhere.

**Consent to participate** All authors agree to be accountable for all aspects of the work.

**Consent to publish** All authors agree with the content and give explicit consent to submit.

**Competing interests** The authors declare that they have no conflict of interest.

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