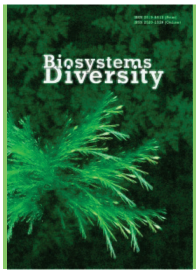

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Estimating biomass of woody plants that grow in the different As-contaminated technosoils in the ore-bearing provinces of Eastern Germany

V. Lovynska*, **, S. Stankevich***, S. Sytnyk*, ****, C. Montzka**,
K. Holoborodko*****, H. Heilmeier******, O. Wiche******

Dnipro State Agrarian and Economic University, Dnipro, Ukraine
Forschungszentrum Jülich GmbH, Jülich, Germany
Institute of Geological Sciences, Kyiv, Ukraine
Czech University of Life Science Prague, Praha, Czech Republic
Oles Honchar Dnipro National University, Dnipro, Ukraine
Technische Universität Bergakademie, Freiberg, Germany

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Dnipro State Agrarian and Economic University, Sergey Efremov st., Dnipro, 49600, Ukraine. Tel.: +38-067-769-63-29. E-mail: glub@ukr.net

Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences: Agrosphere (IBG-3), Jülich, 52425, Germany. Tel.: +49-246-161-32-89. E-mail: c.montzka@fz-juelich.de

Institute of Geological Sciences, Olesia Honchara st., 55-b, Kyiv, 01054, Ukraine. Tel.: + 38-044-482-01-66. E-mail: s.stankevich@nas.gov.ua

Czech University of Life Science Prague, Kamycka, 129, 16500, Praha, Czech Republic. Tel.: +42-072-162-40-75. E-mail: sytnyk@fld.czu.cz

Oles Honchar Dnipro National University, Gagarin av., 72, Dnipro, 49010, Ukraine. Tel.: +38-066-795-63-20. E-mail: goloborodko@ua.fm

Technische Universität Bergakademie, Institute of Biosciences, Leipziger st., 29, Freiberg, 09599, Germany. Tel.: +49-037-313-935-62. E-mail: oliver.wiche@umweltbuero-wiche.de

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Establishing the role of woody species as an instrument for heavy metal bioaccumulation is a relevant issue today in the context of the development of the phytoremediation system. The article presents the results of studies on the influence of different Arsenic (As) concentrations in soil on the development of aboveground biomass in *Betula pendula* Roth. and *Populus tremula* L. stands under conditions of reclamation plantings. The studies were conducted in 30 locations of birch and poplar tree plantations within the ore-producing regions of Saxony (Eastern Germany) in soil with different levels of As contamination. The highest As content was noted in the technosoil of the Davidschacht site, where the metalloid content was 229.3 times greater compared with a value in a conditionally uncontaminated area (Großschirma). The values of leaf area index and aboveground biomass obtained in field measurements were presented. The aboveground biomass values in the investigated plantations ranged from 189.9 ±10.16 to 201.8 ± 19.09 t/ha, and leaf area index values ranged from 1.74 ± 0.29 to 2.05 ± 0.16 m^2/m^2. Sentinel-2A multispectral images were processed for the construction of a map of the aboveground biomass distribution within the region under study. The values of the spectral indices for leaf area index were obtained with subsequent construction of the regression dependence of the aboveground biomass in the plantings on this indicator. The RMSE value for the developed model of the dependence of aboveground biomass on the leaf area index was 17.84 t/ha, which could be considered as satisfactory and can serve as a basis for practical application of the model developed. The inverse trend in relation to locations with different levels of soil contamination with As was determined for the aboveground biomass indicator. Within the region under study, the highest value of aboveground biomass in the stands was found for the area with the lowest As level. The results showed that the correlation coefficient between the highest of the optimal spectral indices, the leaf area index, and the aboveground biomass in *B. pendula* and *P. tremula* plantings was statistically significant and approached the value of 0.7. The results presented can become a theoretical basis for monitoring the accumulation of aboveground biomass of tree stands in areas with different levels of soil contamination with As. In perspective, the presented model of biomass estimation based on spectral technologies can serve as an application basis for rapid assessment of the growth and development parameters of forest stands in As-contaminated areas.

Keywords: metalloids; leaf area index; aboveground biomass; reclamation plants; multispectral images; *Betula pendula*; *Populus tremula*.

Introduction

Studies of the deposition and long-term accumulation of toxic elements in the aboveground biomass of phytocenoses are of considerable theoretical and applied interest, because plants, being autotrophic producers in ecosystems, play an important role in the redistribution and migration of chemical elements between the structural components of biogeocenoses (Fernández et al., 2017; Kozak & Brygadyrenko, 2018; Roque-Alvarezah et al., 2018; Drzewiecka et al., 2019a).

Aboveground biomass is an extremely important parameter in forest and agricultural research for monitoring and predicting the state and growth of plant communities, as well as for obtaining information on yield and the possibility to apply management decisions in good time (Sytnyk et al., 2017; Zhukov et al., 2021; Abhijith et al., 2022; Pidlisnyuk et al., 2022). Since woody plants are a renewable energy resource, it is obvious that providing actual information on the aboveground biomass accumulation can ensure timely management of ecosystems regarding their productivity both at the local and national levels.

Arsenic (As) is an important metalloid that ranks 20th in natural abundance (Wuana & Okieimen, 2011; Demirayak et al., 2019). At the same time, despite its insignificant prevalence, As is very harmful being contained in significant proportion in water-insoluble forms in many plant tissues (Koch et al., 2000). According to some authors, As inhibits plant growth because it affects the absorption of other nutrients (Budzyńska et al., 2019c; Gąsecka et al., 2021). As a result, metabolic processes, primarily nutrient transport, turn out to be significantly altered (Gomes et al., 2012). This has an unfavorable effect on major physiological and biochemical processes, in particular, on aerobic phosphorylation and protein function (Nagajyoti et al., 2010). Along with this, As itself is toxic to plants, animals, microorganisms and humans, especially when its concentration reaches toxic levels in the environment (Moreno-Jiménez et al., 2012; Gąsecka et al., 2021).

Today, it is extremely relevant to study the processes of As removal from contaminated soils with the participation of woody plants that are able to remove this metalloid from the circulation for quite a long time (Alekseenko et al., 2017). The predominant amount of this metalloid accumulates in root systems (Bergqvist et al., 2014; Budzyńska et al., 2019c); however, it is mainly concentrated in plant leaves after reaching aboveground biomass (Łukowski et al., 2020).

Aboveground biomass is a key indicator of the state of plant communities, defining the major criteria for the functioning of forest plantings and their performance of ecosystem services (Algreen et al., 2014). One of the main indicators of the functional state of the assimilation component is considered to be the leaf area index (LAI) directly related to the formation and structure of forest stands (Hikosaka, 2005; Forrester et al., 2017). Vertical leaf density profiles and the total leaf surface area, the proportion of gaps, the height of the forest canopy all affect the rate of gas diffusion inside and out of the tree canopy and the processes of plant biomass formation (Fotis et al., 2018; Lovinskaya et al., 2018). The accumulating characteristics of woody plants should usually be identified in the conditions of ore-bearing provinces, in the soils where an excess of chemical elements is an additional factor for the background content of metals/metalloids, which leads to the formation of both high hyperaccumulative capacity and tolerance in plants (Magdziak et al., 2020; Stefan et al., 2022).

Historically, the Freiberg region in Lower Saxony had significant deposits of silver and arsenic ores, which causes excess percent abundance of metallic elements in soils (Richert et al, 2017). The creation of forest plantings from tree species with significant accumulation potential as a method of soil reclamation has a sufficient history in this region (Fritz et al., 2017; Wiche et al., 2017). Substantiation of the reclamation toolkit and research of the biotic productivity of woody species in the region under study involves determining the selective As-accumulative potential of plants and elucidating their role in the migration and deposition of this metalloid in aboveground phytomass (Midula et al., 2017).

The goal of the work was to determine the features of the aboveground biomass formation in reclamation stands of *Betula pendula* Roth. and *Populus tremula* L. in soils with various levels of As contamination.

Materials and methods

Study area. The study was conducted in 21 locations situated in Lower Saxony, Germany (50°54′46″ N, 13°20′29″ E) covering approximately 1000 km^2 in the southern part of the Central Saxony District. Freiberg lies on the northern declivity of the Ore Mountains; the major part of the region is located to the west of the Freiberger Mulde River. Some parts of the town are located in the valleys of the Münzbach and Goldbach streams. The center has an altitude of about 412 m above sea level. Climatically, the study area is situated in the temperate, humid, continental climate zone of Central Europe and has hot summers and cold, humid winters. The study was conducted in the summer-autumn period of 2022. In order to analyze the input data, the studied locations were grouped into five regions according to their location and in accordance with the level of anthropogenic load (Saxonian State Agency for Environment, Agriculture and Geology). The following names of the studied locations were introduced: Davidschacht, Halsbruken, Großschirma, Freiberg area and Klingenberg (Fig. 1).

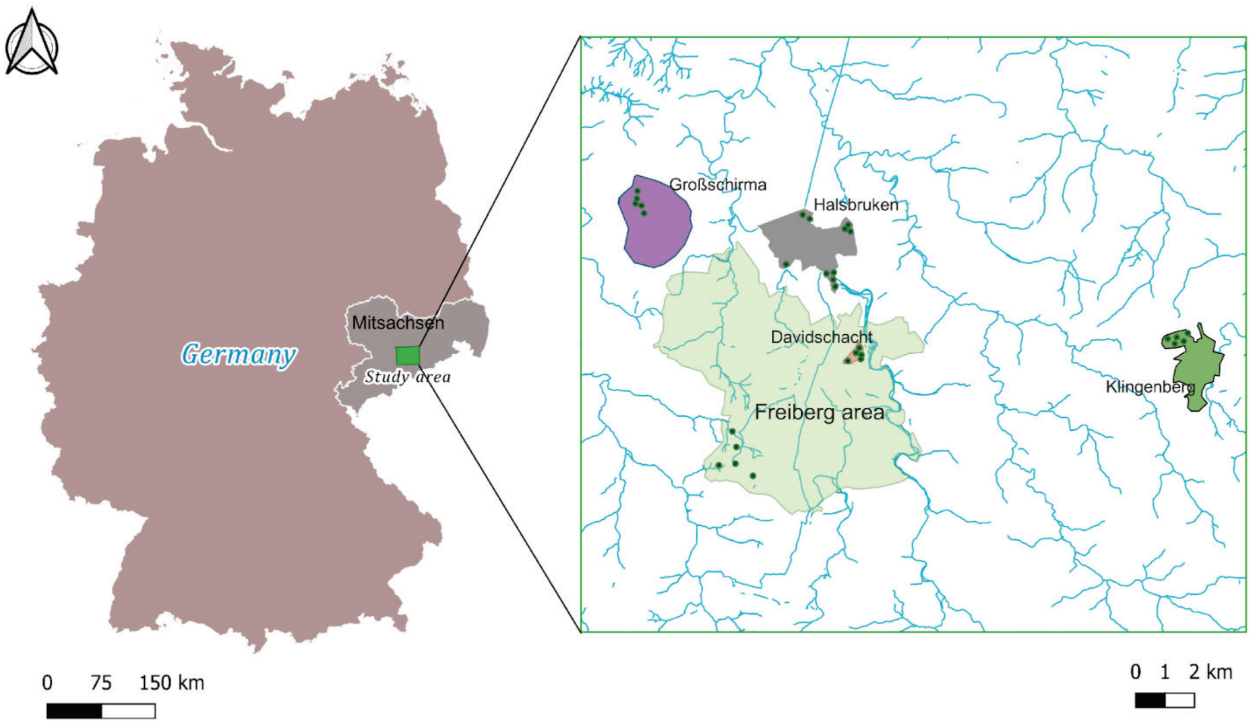

Fig. 1. Study area with the investigated locations

Of the five locations under study, Davidschacht is a historical center of silver ore mining; it is an area covered with numerous mine or slag dumps. The Davidschacht mining dump is located in the municipal area of Freiberg (Fig. 1). It is located near the Freiberger Mulde River (westerly) and on the eastern edge of the city (50˚55′34″ N 13˚22′8″ E). The Davidschacht area covers approximately 72,500 m^2, and its height is in the range of 25–30 m (Fritz & Jahns 2017; Richert et al., 2017).

Field data collection was conducted in 30 sample plots during August 2022 for biomass estimation in the plantation spontaneously formed by *B. pendula* and *P. tremula*. Within each allocated rectangular area (20 × 20 m), a list of tree species, the diameter and height of each tree, the density, and the relative density of plantings were determined (Table 1). In accordance with methods generally accepted in forest taxation (Lakida et al., 1996), the biomass of the forest stand was calculated with the methods used in the previous researches (Sytnyk et al., 2017; Lovynska et al., 2018) and indicators of wood density of trees (birch 0.52 t/m^3, poplar 0.40 t/m^3). Evaluation of the leaf area index in a field condition was carried out indirectly, using the device LAI-2000, Plant Canopy Analyzer. The measurements were performed in 5 points (angular and central) of each of the locations studied. In order to establish leaf area index, the data obtained was further processed with Gap Light Analyzer (Version 2.0) software. Results of leaf area index measurements are given in Table 1. As the aboveground studies have shown, leaf area index values ranged 1.74 ± 0.29 to 2.05 ± 0.16 m^2/m^2.

Table 1
Biometric measurements of the trees within plantations, leaf area index and total aboveground biomass

Location, number of plots	Mean density, number of stems	Mean diameter at breast height, cm	Mean height, m	Leaf area index, m^2/m^2	Aboveground biomass, t/ha
Davidschacht (n = 5)	177	10.4	15.1	1.84	189.9
Halsbruken_1 (n = 10)	203	8.6	18.6	2.01	195.1
Großschirma (n = 5)	243	7.1	17.1	2.04	201.8
Freiberg area (n = 5)	210	7.6	15.0	1.96	190.4
Klingenberg (n = 5)	321	4.1	16.2	2.05	193.1

Estimation of plant biomass with remote sensing methods. As is known, aboveground biomass of vegetation is closely related to the leaf area index, which is the most important phytometric parameter of forest ecosystems (Lu et al., 2016). In remote sensing, numerous methods have been developed for determining leaf area index by multispectral satellite imagery (Zheng & Moskal, 2009).

The modern European Sentinel-2 multispectral satellite system is especially suitable for determining leaf area index (Askar et al., 2018). The SNAP software provided by the European Space Agency (ESA) for Sentinel-2's data handling includes a special Biophysical Processor capable of calculating leaf area index in automatic mode (Xie et al., 2019).

The SNAP Biophysical Processor was used in this research to obtain the leaf area index maps of the study area. The developed leaf area index maps were put in correspondence with ground data of measured biomass, as it was done in Zaitseva et al. (2021). Then nonlinear regression dependence of aboveground biomass on leaf area index was developed as 217.0–44.1/LAI. The equation is valid for LAI >= 0.203 (for LAI < 0.203, take LAI as 0.203). Regression dependence was restored using the SciLab open-source software for numerical computation (Fig. 2).

Ground-based measurements were carried out on August 9, 16, and 24, 2022; they were reconciled with Sentinel-2 satellite images for July 25, August 16 and 29, 2022. The regression error (RMSE) was 17.84 t/ha, which was acceptable. Three Sentinel-2 images used were combined into a single cloudless composite, to which nonlinear regression was applied using SNAP Band Mathematics and as a result, a map of aboveground biomass distribution within the study area was built.

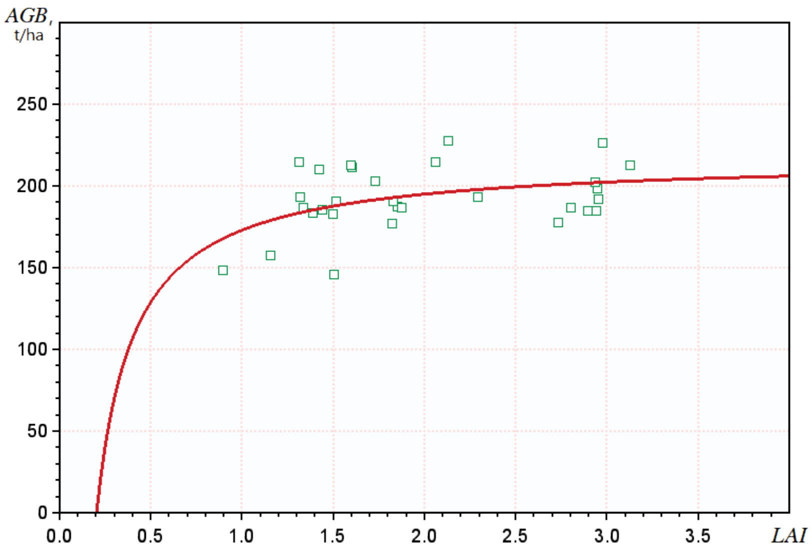

Fig. 2. Nonlinear regression dependence of aboveground biomass (AGB) on leaf area index (LAI) in the plantation formed by *B. pendula* and *P. tremula* within ore-bearing provinces of Eastern Germany

At each experimental site, soil samples were taken from the topsoil (0–20 cm) in an area of 10 m^2 (Ullrich et al. 1999) using a steel ground drill, diameter 3 cm. The mass of the pooled sample was about 1 kg. To estimate As concentrations, soil samples were dried at 105 °C to a completely dry state and ground in a boron carbide solution. 500 mg of the powder was then weighed in a nickel crucible and mixed with 1 g of Na_2CO_3 and 1 g of K_2CO_3. The soil sample was melted for 30 minutes in a muffle furnace at 900 °C. The decanted samples were dissolved in 50 mL of 2 M HNO_3 and 0.5 M citric acid and were measured with inductively coupled plasma mass spectrometry (ICP-MS, Model X Series 2, Thermo Fisher Scientific, Dreieich, Germany). Concentrations of As were determined with ICP-MS (XSeries 2, Thermo Scientific) using 10 µg/L rhodium and rhenium as internal standards. Calibration solutions (0.01–100 µg/L) were prepared by appropriate dilution of a multi-element base standard solution (Petruzzelli et al., 2020).

According to the indicators measured, the greatest As concentration was noted in the soil substrate of the Davidschacht location, while the lowest concentration was found for the conditionally pure Großschirma location, with an excess of the specified indicator in technosoil by 229.3 times.

Differences in the results of As content, as well as biometric indicators of tree stands in soils with different arsenic concentrations, were determined by univariate variance analysis (ANOVA) followed by the HSD Tukey at the significance level of $P < 0.05$ using IBM SPSS Statistics 26 software. Prior to the analysis, the data were tested for uniformity of variance using the Leuven criteria.

Table 2
Total As concentration in the soil of investigated location in the ore-bearing provinces, Eastern Germany (µg/kg, x ± SD, n = 30)

Location	Mean ± standard deviation
Davidschacht (n = 5)	8978.3 ± 509.0^a
Halsbruken (n = 10)	155.4 ± 3.9^b
Großschirma (n = 5)	39.2 ± 4.9^c
Freiberg area (n = 5)	99.8 ± 6.7^d
Klingenberg (n = 5)	111.4 ± 12.4^d

Note: different letters indicate values that reliably differed from one another within the Table according to the results of comparison using the Tukey test with Bonferroni correction.

Results

The results of data on aboveground biomass for all observed plots are given as summary statistics in Table 3. The range of values of this indicator was shown to be significantly narrower and fluctuate from 189.9 to 201.8 t/ha. The lowest value was fixed in the Davidschacht location, while the highest value was found in the Großschirma location. The coefficient of variation was less than 10% for almost all investigated locations except for Freiberg and Halsbuken.

The results of the Tukey analysis showed the homogeneity of the analyzed groups relative to all the analyzed locations. However, the data obtained with this method were not statistically significant.

Table 3
Summary statistics for aboveground biomass (t/ha) in *B. pendula* and *P. tremula* plantations growing in the different investigated locations of the ore-bearing provinces of Eastern Germany ($x \pm SD$, n = 30)

Location	Mean ± standard deviation	Coefficient of variation, %	Minimum-maximum
Davidschacht	189.9 ± 10.2^a	5.35	177.5–202.2
Halsbruken	193.4 ± 23.5^a	12.15	157.7–214.4
Großschirma	201.8 ± 17.1^a	8.47	184.9–226.1
Freiberg area	190.4 ± 32.4^a	17.02	145.7–227.9
Klingenberg	193.1 ± 9.8^a	5.09	185.3–209.8

Note: same letters indicate values not differing within the Table according to the results of comparison using the Tukey test with Bonferroni correction.

The coefficients of correlation calculated on the basis of mean values of aboveground biomass in plantation, As content in soil and leaf area index showed moderately strong relationships only between leaf area index and aboveground biomass when all study sites were considered (r = 0.68, P < 0.0001). At the same time, we did not find significant correlations between As content and indicators of the leaf area index (r = –0.28, P = 0.141) and aboveground biomass (r = –0.17, P = 0.373) in the plantations studied. However, as the data obtained show, clear trends of inverse correlation dependencies were observed in this case. Increasing As content in the soil results in a decrease in the aboveground biomass of the plantations.

Based on the data obtained from aboveground measurements, the dependence of aboveground biomass on leaf area index was obtained and the following allometric equation was derived (Fig. 3).

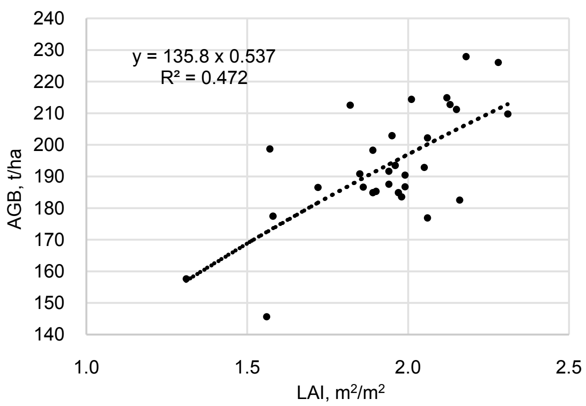

Fig. 3. Dependence of aboveground biomass on leaf area index in *B. pendula* and *P. tremula* plantations in the different investigated locations of the ore-bearing provinces of Eastern Germany (n = 30)

The assessment of the data on aboveground biomass and leaf area index received from Sentinel-2A (S2A) on investigated species in various locations was performed using a generalized linear model. The significance level of the developed model of aboveground biomass dependence in the plantations on leaf area index compared to S2A leaf area index was much greater (Table 4). At the landscape level, the general linear model explains 67% and 33% of the overall variation in biomass and S2A leaf area index, respectively.

As can be seen from the data provided, the results of fitting a general linear statistical model relating S2A leaf area index to leaf area index, there is not a statistically significant relationship between S2A leaf area index and the predictor variables at the 95.0% confidence level. The adjusted R^2 statistic, which is more suitable for comparing models with different numbers of independent variables, was 32.7%. Since the p-value is greater than 0.05, there is no indication of serial autocorrelation in the residuals.

Table 4
General Linear Models analysis of the effect of leaf area index on the S2A leaf area index and aboveground biomass in *B. pendula* and *P. tremula* plantations in the different investigated locations of the ore-bearing provinces of Eastern Germany

Parameter	F-Ratio	P	R_{adj}^2
Aboveground biomass	3.37	0.1225	0.67
S2A leaf area index	1.56	0.3605	0.33

The results of comparing the observed and predicted aboveground biomass and S2A leaf area index are depicted in Figure 4.

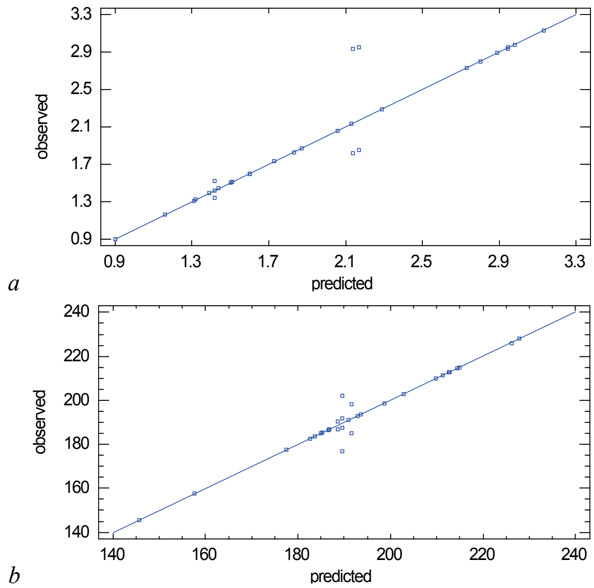

Fig. 4. Observed and predicted values of leaf area index (*a*) and aboveground biomass (*b*) in *B. pendula* and *P. tremula* plantations growing in the different investigated locations of the ore-bearing provinces of Eastern Germany, based on the use of general linear model (n =30)

Predicted values were generated from the regression model utilizing all 30 sample sites. On average, the aboveground biomass estimates for both prediction indexes were slightly higher than the values obtained for the sample plots. Overall prediction bias in aboveground biomass was negligible, with mean absolute value 1.55 t/ha for the general linear model.

Discussion

Arsenic belongs to the group of chemical elements of medium absorption intensity. It is rather poorly accumulated by plants. In plant bodies, As content can vary from 0.009 to 1.5 mg/kg (Kabata-Pendias, 2011). Percent As abundance in plants is 0.1–0.2 mg/kg (Moreno-Jiménez et al., 2012). The root system is the main route of As access to the plant body; through the roots As enters the aboveground biomass. The availability of As contained in the solid soil phase may be limited by arsenate ions bound to iron, calcium, magnesium, and aluminum (Alekseeva et al., 2017; Budzyńska et al., 2019b). According to some reports, As concentration in plants growing in uncontaminated soils is within the range from 0.01 to 5.0 mg/kg (Bergqvist et al., 2014). By the literature data, As concentration that does not affect the normal plant growth and development is 1.0–1.7 mg/kg. Concentration of As from 5.0 to 20.0 mg/kg is toxic (Kabata-Pendias, 2011). The critical As concentration 20 mg/kg in the leaves of woody plants reduces their productivity by 10%. Meharg et al. (2002) assessed As phytotoxicity as strong. The toxic effect of Arsenium is defined by its ability to compete with vital elements, iron and phosphorus.

Growing woody plants in the areas highly contaminated by heavy metal(loid)s (besides the production of biomass intended for energy purposes) could also fulfill the role of land reclamation-gradually purifying the soils from these metals.

Since remote sensing methods are increasingly being used along with the classical methods of estimating the biomass of tree stands (Wei et al., 2020), one of the tasks of our work was to create a map with the predicted distribution of aboveground biomass of woody plants in the region studied. For this, we used the indicators of leaf area index obtained with field measurements as an input parameter for calculating the predicted biomass indicators. It is known exactly which leaf area index is one of those indicators correlated positively with aboveground biomass (Zheng & Moskal, 2009). Similar to these results, our studies also recorded a significant positive correlation in the case of the ratio leaf area indexand aboveground biomass.

The best correlations between the measurements of biomass and the remote sensing data sets were found with the Sentinel-2 measurements. The three Sentinel-2 images used were combined into a single cloudless composite, to which nonlinear regression was applied using SNAP Band Mathematics and as a result, a map of aboveground biomass distribution within the study area was obtained and represented in Figure 5.

In the studied region, woody species form a biomass in the amount of 60–210 t/ha (Fig. 3). In the studied reclamation plantings, aboveground biomass of woody species capable of accumulating the investigated metalloid within the following ranges (in kg/kg) was equal: in *B. pendula* 0.70 to 1.70, in *P. tremula* 0.63 to 12.0.

Differences in the spatiotemporal distribution of some toxic elements, including As, are important factors in the formation of differences in the distribution of vegetation in terrestrial ecosystems and are of key importance for the development of plant biomass.

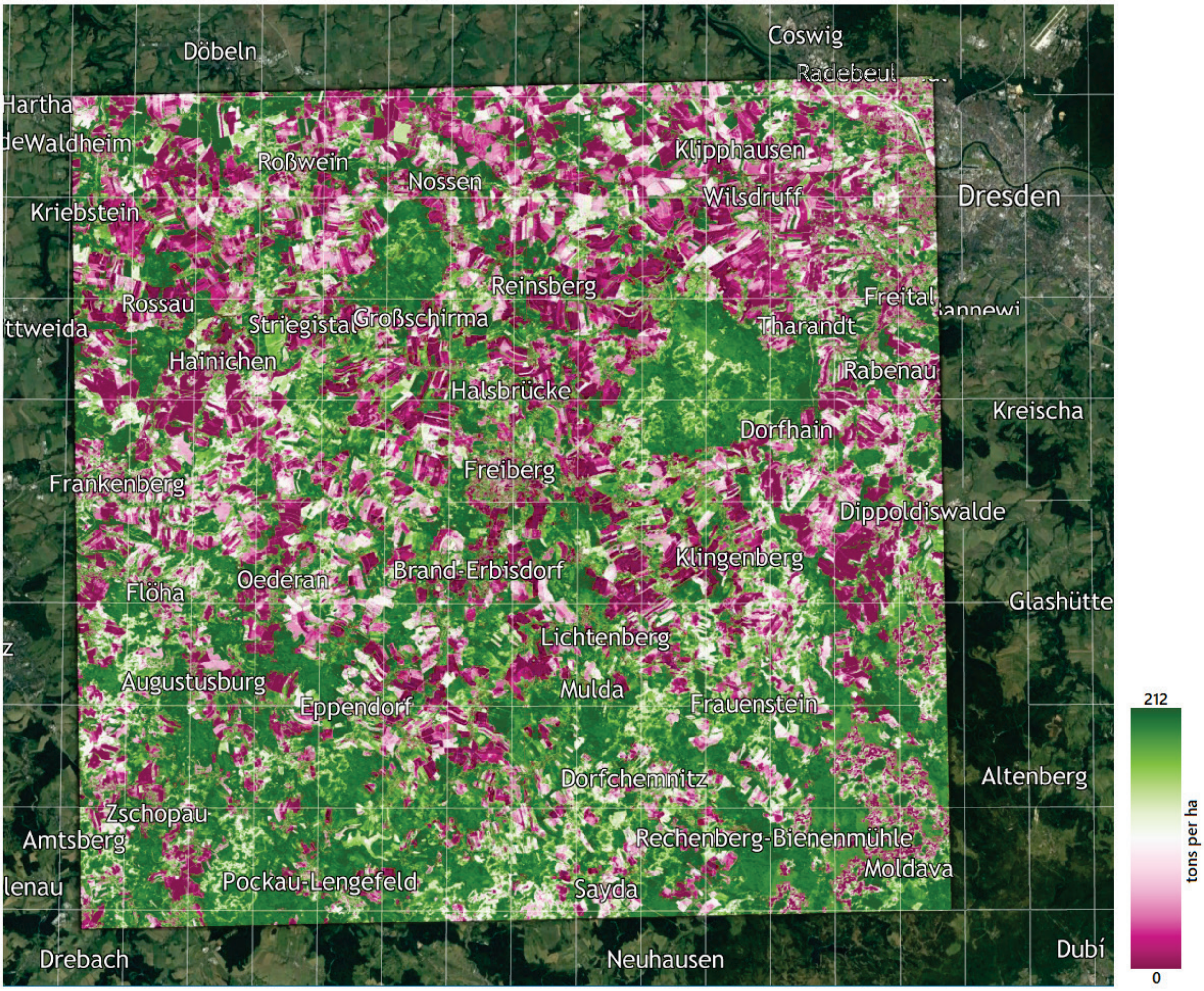

Fig. 5. Aboveground biomass distribution map of the study area

Excess As accumulation in the tissues of vegetative and generative plant organs will affect the processes of photosynthesis, which will have a direct impact on accumulation of the aboveground biomass in the plantings created for phytoremediation of As-contaminated soils.

Seedlings of such woody species as *Acer platanoides* L. and *Tilia cordata* Miller. are able to grow in experimental systems with the addition of As. However, when exposed to tree seedlings in this system with the greatest arsenic content, an overall decrease in plant biomass was observed (Budzyńska et al., 2019b). Similar to the results established by the abovementioned authors, in our experiment, the measured biomass of the three woody species studied that formed "spontaneous" plantings on Davidschacht was also lower compared to the other variants studied. Different arsenic forms are presented simultaneously in soils, for example As(III), As(V) (Takamatsu et al. 1982; Drzewiecka et al., 2019). Arsenic is mainly transported by plants through root uptake, in a process similar to the way nutrients and other trace elements are absorbed (Fitz & Wenzel, 2002). In particular, an analogy is drawn between phosphate and arsenate, which allows us to establish certain parallels between the rhizosphere dynamics of P and As. Since arsenate and phosphate are considered to be chemical analogues, all these processes are likely to mobilize As, for example, organic acids are able to displace arsenate from exchange positions in soils (Wenzel, 2009). In addition, plant strategies aimed at attacking Fe oxide-hydroxides also alter the surfaces on which As is retained, and this can potentially solubilize As (Fitz & Wenzel, 2002).

As can be seen from our results, although the biomass of woody stands growing in As-contaminated soils tends to decrease, the value of the difference with the maximum content (Großschirma) was insignificant (7%). This can be explained by the fact that As transport in most plant species is usually not very efficient, and therefore As tends to be remained in the roots (Moreno-Jiménez et al., 2012). An exception exists for those plants that accumulate As extremely efficiently in their aboveground parts (Francesconi et al., 2002; Mleczek et al., 2017). Arsenic accumulation in vacuoles may be one of the reasons for the decreasing transport to xylem (Zhao et al., 2009).

The correlation data presented in this paper did not reflect a clear significant relationship between biomass indicators and As content accumulated in soils. First of all, this is explained by the fact that the paper presents data on the assessment of aboveground phytomass, and as noted above, As accumulates mainly in the underground plant phytomass. This is confirmed by the authors who studied As accumulation in the underground part of plants and its effect on biomass (Matzen et al., 2022). Thus, the majority of As (>66%) accumulated in *Pteris vittata* was of rhizospheric origin.

Conclusions

The data obtained as a result of the research indicated an uneven distribution of metalloid contents in the technosoil of the investigated territory. Relative to the studied locations, Arsenicum content was distributed as follows: Davidschacht > Halsbruken > Klingerberg > Freiberg area > Großschirma. The results obtained for plant biomass are inversely proportional to Arsenicum content, with the highest value in Großschirma and the lowest in the most polluted area of Davidschacht. Leaf area index values in investigated location varied from 1.84 to 2.04 m^3/m^3; the lowest values of this parameter were found in Davidschacht location. The highest correlations were found between the leaf area index and aboveground biomass values, with a reliable correlation coefficient of 0.68. A regression model for determining the aboveground biomass of fast-growing woody species as *B. pendula* and *P. tremula* was characterized by a satisfactory RMSE of 17.84 t/ha. This makes it possible to further application of the regression developed with the use of the leaf area index as an input parameter and the biomass determination in As-contaminated areas.

The authors declare that there is no conflict of interest.

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