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WHAT IS DRIVING THE EVOLUTION OF THE FAR-INFRARED RADIO CORRELATION?

ABSTRACT: Far infrared-radio correlation represents a linear relationship between far-infrared (FIR) and radio emission in star-forming galaxies. Previous observations have confirmed that this correlation is maintained over a large range of redshift and does not evolve, although a small dispersion is present. However, some of more recent observations at high redshift have shown the opposite. The question that arises is – what is driving this evolution? In this paper we investigate the possibility that galaxy morphology is the answer to this question. A sample of 37 submillimeter galaxies (SMGs) is analyzed. The observation and morphological class of these galaxies has previously been published. We examined FIR-radio correlation in galaxies of different morphological type in this sample and found that for star-forming disk galaxies correlation is stable and does not evolve and for irregular and interacting galaxies we find some hints of evolution.

KEYWORDS: cosmic rays, galaxy evolution, interactions of galaxies, infrared: galaxies, radio continuum: galaxies

INTRODUCTION

By the 1960s a strong linear correlation was established between the farinfrared and radio emission in star forming galaxies (van der Kruit, 1971; Helou et al., 1985; Condon, 1992; Yun et al., 2001), named FIR-radio correlation. It is assumed that the origin of this correlation lies in young massive stars because they are the main source of emission in these two bands. The UV emission that comes from these stars heats up dust which then emits infrared radiation. On the other hand, when massive stars die as supernovae, their remnants become a spot where electrons are accelerated and enhance non-thermal radio emission (Condon et al., 1991). This suggests that correlation can be used as a

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probe of the star formation (SF) process (Condon, 1992; Bell, 2003; Murphy et al., 2012). Other SF tracers that are frequently used such as H α or UV emission suffer dust extinction and corrections for this process bring significant uncertainty. The dust emission (Lisenfeld & Volk, 1993) in the mid-infrared range (typically taken at 24 µm or 70 µm), which is also used to trace the SF processes, is not affected by dust extinction, but these observations need to be done by space-satellite missions (Spitzer, WISE, Herschel) that have limited spatial resolution. The radio emission is not affected by dust, thus avoiding both disadvantages. Another application of the FIR-radio correlation is the use of the radio-submillimeter ratio as a photometric redshift indicator (Blain, 1999). Radio-loud active galactic nuclei can be identified and studied using FIR-radio correlation (Donley et al., 2005; Norris et al., 2006; Park et al., 2008; Del Moro et al., 2013). Also, this correlation is used to estimate the distances and temperatures of high-redshift submillimeter galaxies (Condon, 1992; Bell, 2003; Murphy et al., 2012). The FIR-radio correlation is defined with the ratio parameter

$$q_{\rm FIR} = \log\left(\frac{F_{\rm FIR}}{3.75 \times 10^{12} {\rm Wm}^{-2}}\right) - \log\left(S_1 \cdot \frac{S_{1.4}}{{\rm Wm}^{-2} {\rm Hz}^{-1}}\right)$$
(1)

Where F_{FIR} is dust far-infrared emission flux density from 24µm to 144µm and S_{14} is radio emission flux density at 1.4 GHz (Helou et al., 1985). Multiple studies have claimed that FIR-radio correlation does not evolve with redshifts and remains stable (Sargent et al., 2010), and that it can be described by a single value. Yun et al. (2001) have determined this value from a sample of 1,800 star-forming galaxies to be $q_{\rm FIR} = 2.34 \pm 0.01$. Due to increasing density of cosmic microwave photons with redshift, one should expect evolution of this parameter with redshift. These photons would result in more significant Inverse Compton energy losses by electrons, and decrease in the non-thermal radio emission would appear (Murphy et al., 2009). Recent research carried out on 12,000 star-forming galaxies from COSMOS showed the evolution of $q_{\rm FIR}$ parameter with redshifts $q_{FIR}(z) = (2.52 \pm 0.03) (1 + z)^{-0.21 \pm 0.01}$ (Delhaize et al., 2017), demonstrating that either infrared emission is decreasing or radio emission is increasing more significantly with redshift. Possible contamination by the presence of AGN can be the explanation for this observed trend (Magnelli et al., 2010; Sajina et al., 2008) or larger contribution of thermal radio component (Delhaize et al., 2017). Also, increasing factor of major mergers with redshifts can explain this evolution. Interaction of galaxies can result in additional emission from gas bridges in Taffy-like system (Murphy, 2013) and amplification of magnetic fields (Kotabra et al., 2010). In tidal shocks that form in interstellar medium of interacting galaxies, acceleration of cosmic rays appears (Donevski & Prodanović, 2015). All these phenomena will contribute to the increase of non-thermal synchrotron emission.

Inspired by previous conclusions, in this paper we explore the possibility that major mergers are responsible for the decrease in the q_{FIR} parameter found

in Delhaize et al. (2017). We do this by looking at the impact of morphology on the evolution of FIR-correlation with redshift (Pavlović & Prodanović, 2019). In local universe there is a fraction of galaxies that do not resemble either disk or elliptical galaxies – the irregular galaxies. This morphology is the indication of past or ongoing galactic interaction. The number of irregular galaxies is increasing towards higher redshifts (Mortlock et al., 2013). A small sample of high redshift SMGs that were morphologically classified (Miettinen et al., 2017a, b) will be analyzed in this paper in order to explore changes of FIRradio correlation with morphology.

MATERIALS AND METHODS

The target submillimeter galaxies (SMGs) in this work were first discovered by the $\lambda_{obs} = 1.1$ mm blank-field continuum survey over the area of 0.72 deg² or 37.5% of the full 2deg² COSMOS field conducted with the AzTEC bolometer array on the 10m Atacama Submillimeter Telescope Experiment (ASTE) (Ezawa et al., 2004).

In this paper we analyzed 37 SMG galaxies taken from Miettinen et al. (2017a), 11 of which being tagged as irregular and 26 as disk galaxies, where morphology has already been determined.

Radio and FIR Fluxes

In this study, we used the Herchel continuum observations of FIR (24μ , 100µm, 160µm, and 250µm) to submm (350µm and 500µm) (Pilbratt et al., 2010), which were derived as part of the Photodetector Array Camera and Spectrometer (PACS) Evolutionary Probe (PEP; Lutz et al., 2011). From the SED curve we calculated total FIR flux for each galaxy separately. SED curve was determined from flux density measurements of the infrared emission at 4 wavelengths (24μ , 100µm, 160µm, and 250µm), fitted by the second-degree polynomial:

$$\log S_{\rm FIR} = a + b \log \lambda + c \log \lambda^2 \tag{2}$$

Where *a*, *b* and *c* are the fitting constants, S_{FIR} is the flux density of the infrared emission and λ is the wave length. Integrating the SED curve in the range of 42µm to 122µm, we obtained the total FIR flux density that was then used to calculate the q_{FIR} parameter. The radio flux density at 1.4GHz was taken from the VLA (Very Large Array)-COSMOS survey (Schinnerer et al., 2010; Aretxaga et al., 2011; Miettinen et al., 2017b).

RESULTS AND DISCUSSION

$$S = 10 \times \sum_{x,y=1,1}^{N,N} \frac{(Ix,y - Itx,y) - Bx,y}{Ix,y}$$

Our sample of 37 SMGs (irregular + disk galaxies) has the FIR-radio correlation parameter with mean value of $q_{\text{FIR}} = 2.00 \pm 0.56$, which is lower than the nominal $q_{\text{FIR}} = 2.34 \pm 0.01$ (Yun et al., 2001) but it is within the lower limit.

We divided the sample into two sub-samples, based on their morphology, in order to examine the dependence of the q_{FIR} parameter evolution with redshift for different morphological types, and analyzed them separately. Dependence of the q_{FIR} on z in both cases is fitted by a power-law function $q_{\text{FIR}} = a(1+z)^b$, where a is constant, and b is the degree coefficient. We used fitting function of this type for easier comparison with the result from Delhaize et al. (2017).



Figure 1. q_{FIR} as a function of redshift *z*. Left: blue dash-dotted line represents the fit for 11 irregular galaxies (black triangles). Right: data for 26 disk galaxies (black empty circles) were fitted with red

Figure 1 shows dependence of q_{FIR} on redshift for 11 irregular (left) and 26 disk galaxies (right) from our sample. The blue dash-dotted line and red dashed line show the power-lawfits for irregular and disk galaxies respectively: $q_{\text{FIR}} = (2.45 \pm 0.83)(1 + z)^{-(0.34 \pm 0.32)}$ and $q_{\text{FIR}} = (2.11 \pm 0.54)(1 + z)^{-(0.09 \pm 0.23)}$ (Pavlović & Prodanović, 2019).

Finally, we examined the whole sample together (disk + irregular galaxies) which are shown on Figure 2 with green solid curve in the form $q_{FIR} = (2.05 \pm 0.43)(1 + z)^{-(0.11\pm0.18)}$ (Pavlović & Prodanović, 2019). On the same plot there are also all curves, for disk (red dashed line) and irregular (blue dash-dotted line) galaxies alone, and we compare everything to results found in Delhaize et al. (2017) that are presented with pink curve. As in Figure 1, the black triangles represent 11 irregular galaxies while black empty circles are 26 disk galaxies.



Figure 2. q_{FIR} in a function of redshift for 37 SMGs. Black empty circlesrepresent disk SMGs and black triangles are irregular galaxies. Red dashed and blue dash-dotted lines represent power-law fits fordisk and irregular galaxies, respectively. The green, curve shows power-law fit for the entire sample of 37 SMGs (disk + irregular) galaxies. Pink solid curve is the same power-lawfit found in Delhaize et al. (2017).

CONCLUSION

FIR-radio correlation was proven to be a very good tool for determining SFR (Condon, 1992). Its use is based on the assumption that it remains stable across a large range of redshifts, despite significant scatter. However, recent observation of star-forming galaxies in the COSMOS survey has shown a

decrease of q_{FIR} parameter towards higher redshifts and evolution in the form: $q_{\text{FIR}}(z) = (2.52 \pm 0.03)(1 + z)^{-0.21 \pm 0.01}$ (Delhaize et al., 2017).

In order to explain the observed evolution, and inspired by claims that galaxy mergers might result in cosmic-ray acceleration that affects the FIR-radio correlation (Lisenfeld & Volk, 1993; Murphy, 2013; Donevski & Prodanović, 2015), we have tested the behavior of correlation with respect to galactic morphology (Pavlović & Prodanović, 2019). We used a sample of 37 SMGs that have already been morphologically classified (Miettinen et al., 2017a, b). The sample was split into two sub-samples, 26 disk galaxies and 11 interacting and irregular galaxies. Due to insufficient data used for statistics, trends found for separately analyzed samples showed no evolution. For irregular and interacting galaxies we found evolution in the form of $q_{\text{FIR}} = (2.45 \pm 0.83)(1 + z)^{-(0.34 \pm 0.32)}$, and $q_{\text{FIR}} = (2.11 \pm 0.54)(1 + z)^{-(0.09 \pm 0.23)}$ was found for disk galaxies. The evolution trend for combined sample (disk + interacting galaxies) was found in the form of $q_{\text{FIR}} = (2.05 \pm 0.43)(1 + z)^{-(0.11\pm0.18)}$, which is consistent with Delhaize et al. (2017). In the pure-disk sample results are consistent with no evolution, which indicates that evolution found on the combined sample is dominated by the irregular and interacting galaxy sample. It is noteworthy that our findings of different evolution for irregular and disk morphology is consistent with results of Molnar et al. (2018), who found decreasing IR/radio ratio for spheroid-dominated galaxies and non-evolving ratio for disk-dominated galaxies. Both results could indicate that there are additional processes that contribute to the enhanced radio emission related to galactic interactions and/or AGN activity. In the future, a larger sample will be used for testing these results. We will try to identify systems with ongoing interaction and compare their IR/radio ratio to that in irregular and spheroid systems, which will be important for selecting between possible presence of AGN and some new processes.

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ШТА УЗРОКУЈЕ ЕВОЛУЦИЈУ ДАЛЕКЕ ИНФРАЦРВЕНЕ РАДИО КОРЕЛАЦИЈЕ?

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РЕЗИМЕ: Опште је познато да постоји јака линеарна веза између далеког инфрацрвеног (ДИР) и радиозрачења у галаксијама у којима се формирају звезде. Међутим, неколико скоријих истраживања на већим црвеним помацима показују да долази до одступања од ове корелације. Питање које се само намеће је – шта покреће ову еволуцију? У овом раду истражићемо могућност да одговор можда лежи у морфологији самих галаксија. Анализирали смо узорак од 37 субмилиметарских галаксија, где смо испитивали ДИР-радио корелацију у зависности од морфолошког типа и пронашли да код дисколиких галаксија у којима се формирају звезде, не долази до еволуције корелације, док код неправилних галаксија постоји индикација за сличну еволуцију корелације коју су показала и раније истраживања.

КЉУЧНЕ РЕЧИ: космичко зрачење, галаксије: интеракција, галаксије: еволуција, радио континуум: галаксије, инфрацрвено зрачење: галаксије Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. Novi Sad, № 137, 17—22, 2019

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UNDERSTANDING THE DIFFUSE GAMMA RAY EMISSION OF THE MILKY WAY – FROM SUPERNOVA REMNANTS TO DARK MATTER

ABSTRACT: Diffuse gamma ray emission from the Galactic center at 2–3 GeV, as well as the 12 TeV gamma ray excess in the Galactic disk, remain open for debate and represent the missing puzzles in the complete picture of the high-energy Milky Way sky. Our papers emphasize the importance of understanding all of the populations that contribute to the diffuse gamma background in order to discriminate between the astrophysical sources such as supernova remnants and pulsars, and something that is expected to be seen in gamma rays and is much more exotic – dark matter. We analyze two separate data sets that have been measured in different energy ranges from the "Fermi-LAT" and "Milagro" telescopes, using these as a powerful tool to limit and test our analytical source population models. We model supernova remnants and pulsars, estimating the number of still undetected ones that contribute to the diffuse background, trying to explain both the Galactic center and the 12 TeV excess. Furthermore, we aim to predict the number of soon to be detected sources with new telescopes, such as the "HAWC".

KEYWORDS: diffuse background, gamma rays, supernova remnants, pulsars, dark matter

INTRODUCTION

Our current understanding of the overall gamma ray emission of our Galaxy, the Milky Way, relies a lot on findings from different ground based and satellite telescopes. One of the largest contributors to the gamma ray image of the sky is most definitely the Fermi telescope^{**}, specifically the LAT part of

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^{**} The official Fermi Telescope page: https://fermi.gsfc.nasa.gov

the satellite that provided us with the best whole-sky gamma-ray image. From these findings we are able to categorize the emission into several contributing categories – point sources (Galactic and extragalactic), Fermi bubbles and the diffuse emission. If we compare everything measured by the instruments with the models of sources we have, ideally, we should be able to account for the entire gamma-ray emission. The reality is somewhat different – we can discriminate between the modeled Fermi bubbles and resolved point sources, as well as modeled diffuse emission, but after subtracting all these, what we are left with is the unresolved diffuse background – a collection of still unresolved sources and potential exotic contributors such as dark matter.

The Galactic diffuse emission is mostly produced in the interactions of cosmic rays in the interstellar medium (ISM). Another potential sources of gamma rays in the Galaxy could be the illusive dark matter. Some of the mainstream theories of what exactly dark matter is allow for a process of self-annihilation of dark matter particles, in places of sufficient densities, which could produce gamma rays of different energies depending on the mass of the annihilating particles. This signal, if existing, should be mixed into the diffuse background, and rather weak one, having in mind that the self-annihilating process is rare. What is fortunate is that there are indications where we should look for such a signal. Places where dark matter gamma ray signal should be most likely detected are places of the largest dark matter densities, and one of such places is the Galactic Centre (GC) (Petrovic et al., 2014).

Indirect dark matter searches have been fueled by better understanding the diffuse emission and the data collected by Fermi LAT. In 2014, Daylan et al. (Daylan et al., 2014) published a paper claiming they have found a spherically symmetric signal from around the GC (1.5 kpc), that peaks at 1–3 GeV that could be fitted with a dark matter map with a 40 σ significance. This initiated a series of papers devoted to what was named the "GC excess" that gave other possible explanations such as a bursting source and millisecond pulsars (Petrovic et al., 2014, 2014). The searches continued and were even spread to our neighboring galaxy – M31, which seems to show a similar signal around its center (Eckner et al., 2017).

What has become obvious is that the indirect dark matter searches have two prerequisites for success – new telescopes with better angular and energy resolution and deep understating of all of the other diffuse gamma contributors. Our aim is to study source populations of different kinds that all contribute to the diffuse background and could thus resent a foreground to the dark matter signal, and also giving explanations to the open questions such as the GC excess and the Milagro TeV excess, which will be discussed in detail in the next section.

METHODS

As previously mentioned, there are still open questions when considering the diffuse gamma ray emission of the Mliky Way. One such issue has been the Galactic center excess problem that still remains open for debate and awaits better telescopes that could give the final verdict whether these gamma rays come from astrophysical sources such as millisecond pulsars or dark matter.

Another rather interesting topic is the Milagro TeV excess, a still unexplained high energy flux of gamma rays equaling to 23.1±4.5×10⁻¹³ TeV⁻¹ cm⁻² s⁻¹ sr⁻¹ 12 TeV, that has been measured by the Milagro telescope in the Galactic coordinate region $-2^{\circ} < b < 2^{\circ}$, $30^{\circ} < l < 65^{\circ}$ (Abdo et al., 2008).

This excess was first detected in the light of EGRET data in 2006 (Prodanovic, 2006). We have now used the latest Fermi LAT data to see if the situation has changed and if the excess is still present and cannot be accounted for by the known and expected source populations from the LAT 4FGL^{*} catalogue - the latest Fermi catalogue of point sources.

One of the issues when considering a high-energy excess such as the Milagro one, is the fact that the Fermi LAT diffuse model does not reach such high energies and has to be extrapolated to check whether it can explain the 12 TeV point, emphasizing the necessity for telescopes that can operate in the high energy mode. After extrapolation, the excess remains present with a flux of 4×10^{-12} photons TeV⁻¹ cm⁻² s⁻¹ sr⁻¹. We have also tested the measured flux against the point sources that have been resolved and are available in the Fermi LAT and TeVCat^{**} catalogues, covering the GeV and the TeV energies, while also conducting a search for GeV-TeV point source counterparts. Even after all of the known GeV and TeV sources are subtracted, along with the diffuse emission we have from the Fermi LAT, the 12 GeV data point remains incompletely explained.

RESULTS

Our idea is to model populations of unresolved astrophysical sources such as pulsars, pulsar wind nebulae (PWN) and supernova remnants (SNR) that could potentially explain this excess measured by Milagro as they are sources of gamma-rays in our Galaxy. These sources are potentially still undetected and remain hidden and contributing to the diffuse background, which is why they are not present in current catalogues.

The number of such sources can be derived from the spatial distribution that is constructed based on current observational and theoretical data for all relevant source populations - SNRs, PWNs and pulsars. Here it should be noted that PWNs and pulsars can practically be treated as one source category as they follow the same distribution and PWN, of course, cannot exist without their fueling mechanisms which are pulsars (Fermi LAT collab. & PTC 2010).

We can calculate the number of potential sources in the Milagro regionof-interest (ROI) where the excess is present, and check whether these can potentially account for the excess emission. For example, for SNR we use the exponential spatial distribution (Green, 2015):

^{*} https://heasarc.gsfc.nasa.gov/W3Browse/fermi/fermilpsc.html ** http://tevcat.uchicago.edu

$$f_{SNR}(r) = \left(\frac{r}{r_*}\right)^{\alpha} exp\left(\frac{r-r_*}{r_*}\right)$$
(1)

where γ is the galactocentric distance, and γ_* is the galactocentric distance of the Sun (8.5 kpc), and a power-law flux with an exponential cut-off (E_{cut}) at 12 TeV (Fermi-LAT collab. 2019):

$$F \propto E^{-r} \exp\left(\frac{E}{E_{cut}}\right)$$
 (2)

to derive a total gamma-ray flux of all potentially unresolved SNRs in the Milagro ROI and compare it to the measured Milagro flux, as well as the diffuse gamma-ray background from Fermi LAT and all known point sources. We can calculate the given population flux if we know the luminosity function of a certain population or if we treat the luminosity as a parameter of a certain value. For this value we choose the maximal and minimal luminosities of known and measured SNRs to have the broadest picture of this source population (Fermi-LAT collab. 2019). What we use as a free parameter is the Γ spectral parameter that can vary from source to source. We limit the variation from 1.5 to 2.5 which are the limits in which most of the SNR Γ -value resides (for sources that appear in catalogues such as the 4FGL).



Figure 1. Modeled emission of the unresolved population of SNRS of maximal luminosity residing in the Milagro region of interest. We show the total population gamma ray flux compared to the Fermi measurements up to 50 GeV (red dots), extrapolated to high energies (red dashed line) and the Milagro excess data point (blue). We vary the spectral index of the population from 1.5 to 2.5.

On Figure 1. we present a population of SNR in the Milagro ROI (multi-color lines) with a given spatial and spectral distributions, as well as the Milagro data point and the Fermi diffuse background measurements (data points up to 50 GeV and high-energy extrapolation) in red. The luminosity is of the order of 10^{36} GeV s⁻¹.

As can been seen in the Figure 1. such a population of SNRs can successfully reach the Milagro data point and potentially explain this excess in gamma rays for values of the spectral index on the lower limit end (1.5-1.9), without disrupting the existing image of the diffuse background measured by Fermi LAT.

DISCUSSION

Next steps involve theoretical predictions for other mentioned sources – pulsars and PWNs and seeing how they compare to the detected Milagro flux.

What can be added into consideration is how many of these potentially undetected sources can, and possibly will be, detected by the newest telescopes commissioned to look at the wide energy range gamma-ray sky. One such telescope is the HAWC telescope^{*} that covers the 100 GeV – 100 TeV energy range, thus being a great candidate to solve the Milagro excess mystery. By using the angular (0.1° for energies > 10 TeV) and energy resolution (below 50% above 10 TeV) of HAWC we can predict the number of postulated sources that can be seen by HAWC. This is the topic of our future work that is currently in preparation and can hopefully give us more insight in what is the actual nature of this excess.

CONCLUSION

From what can be seen in this overview, it is clear that the next step needs to be employing better gamma ray detectors both sky and ground based. The HAWC telescope is a big leap forward and after it has collected a substantial amount of data we should we able to draw better conclusions on what the GC excess and Milagro excess actually are.

There is a need for detectors with better both angular and energy resolution that can both peer into the GC at low energies, but also do all sky observations on energies that range from a few GeV to a few tens of TeV in order to get a complete picture of the gamma ray sky.

We need to understand all the astrophysical sources to the best of our abilities in order to be able to disentangle them from the long awaited dark matter signal.

^{*} https://www.hawc-observatory.org

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РАЗУМЕВАЊЕ ДИФУЗНЕ ЕМИСИЈЕ ГАМА-ЗРАЧЕЊА МЛЕЧНОГ ПУТА – ОД ОСТАТАКА СУПЕРНОВИХ ДО ТАМНЕ МАТЕРИЈЕ

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РЕЗИМЕ: Дифузно гама зрачење из центра наше галаксије на енергијама од 2–3 GeV, као и вишак зрачења на 12 TeV у региону галактичког диска још увек су отворена питања која нас деле од комплетне слике високоенергијског зрачења Млечног пута. Наши радови акцентују битност разумевања свих популација извора који доприносе овом зрачењу у циљу разликовања астрофизичких извора попут пулсара и супернових и нечега што би такође требало да остави отисак на гама небу – а то је тамна материја. Анализирамо податке са "Fermi-LAT" и "Milagro" телескопа, где помоћу два сета мерења у два различита енергијска опсега лимитирамо и тестирамо наше аналитичке моделе различитих популација. Моделујемо пре свега остатке супернових, пулсаре и милисекунд пулсаре, те процењујемо који број наведених извора још увек измиче детекцији и учествује у неразлученој гама позадини, истовремено покушавајући да дамо одговор шта то сачињава вишкове зрачења у центру галаксије и на 12 TeV у диску. Такође, циљ нам је и предвиђање броја детектованих извора у блиској будућности помоћу нових телескопа какав је телескоп "HAWC".

КЉУЧНЕ РЕЧИ: дифузно гама позадинско зрачење, гама-зраци, остаци супернових, пулсари, тамна материја

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EXAMINING THE MOPHOLOGY OF GALAXIES AT HIGH REDSHIFTS

ABSTRACT: Irregular galaxies are considered to be results of collisions or close approaches between galaxies. In the local universe, determining the morphological type and collision stage does not pose a problem. However, when it comes to galaxies at high redshifts, determining morphology is non-trivial. In this paper, the morphological parameters used to determine the morphology of galaxies at large redshifts will be summarised and described in detail. The aim of this research is to examine the sensitivity of morphological parameters at different collision stages on galaxies in the Local Universe, so that the most sensitive parameter can be found and later applied to the study of collisions between distant galaxies. KEYWORDS: galaxy morphology, high redshift, morphology parameters

INTRODUCTION

Increasing number of galactic imaging surveys such as Hubble Space Telescope (HST) Ultra Deep Field (Beckwith et al., 2006), Sloan Digital Sky Survey (Stoughton et al., 2002), or COSMOS (Scoville et al., 2007b) resulted in the need for automated classification methods. These methods should classify large numbers of galaxies faster than any visual examination, and should also be less subjective.

One of the most significant aspects of galaxy study is the evolution of their structure over cosmic time. Many imaging surveys reveal a large amount of irregular galaxies that do not fall into the Hubble classification scheme. This can be especially noticed at higher redshifts due to the fact that more distant galaxies are seen at a significantly younger stage, in which starburst events and mergers occur at a larger rate (Madau et al., 1998; Bell et al., 2006a).

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Inspired by these challenges, new parameters that describe structure have been developed – the concentration index (Conselice, 2003), the asymmetry index (Conselice, 1997) and the clumpiness index (Conselice, 2003). These are known as the CAS parameters. Two other parameters that are frequently used are the Gini coefficient (Lotz et al., 2004) and the M_{20} parameter (Lotz et al., 2004). Gini describes the distribution of flux values among the pixels of an object's image, while M_{20} quantifies the distribution of the brightest 20% of galactic pixels. The difference between CAS parameters and G and M_{20} is that neither Gini nor M_{20} require the center of a galaxy to be defined, and thus do not need to have a well-defined visible center.

One application of these measures was the identification of galaxy mergers. Studying their abundance and properties is crucial to understanding the origin of the most massive galaxies in the universe today (Bell et al., 2006b).

Other particularly interesting application could be the classification of galaxy mergers by stages which can help us understand how the merger process proceeds and furthermore may help in identifying the presence of tidal cosmic rays, accelerated in tidally interacting galaxies (Donevski & Prodanovic, 2015).

Lotz et al. (2006) studied the fraction of major and minor merger candidates among the high-redshift star-forming galaxies, using classification based on Gini, M_{20} , and concentration. A major merger selection criterion was established by Lotz et al. (2008a) using the two-dimensional parameter space of Gini (G) and M_{20} with $G > -0.14M_{20} + 0.33$, enabling them to study the evolution of the galaxy merger rate (Lisker, 2008).

In this paper, we present these parameters, their correlations, as well as their potential use in our future work.

OVERVIEW OF METHODS

Concentration Parameter

Concentration parameter (C), which is used to quantify central density of galaxy light distribution, is calculated using the following equation (Conselice, 2003):

$$C = 5 \times \log\left(\frac{r_{80}}{r_{20}}\right)$$
(1)

where r_{80} represents the radius of the area which contains 80% of galactic light, while r_{20} represents the radius of the area which contains 20% of galactic light. In other words, each radius represents semi-major axis of the ellipse which contains a certain part of total amount of light.



Figure 1. Top: Image concentration (C) vs. B–V color index. Bottom: 180° rotational image asymmetry (A) vs. B–V color index (Bershady et al. 2000)

It was shown that there are strong correlations between spectral index and form parameters describing concentration and asymmetry (Figure 1). We see that early type galaxies are located towards the red end of the spectrum and exhibit larger concentration values, unlike intermediate and late type galaxies, which are closer to the blue end of the spectrum and have generally lower concentration.

Measurements also show that galaxies which have higher concentration also tend to have lower asymmetry and higher mean surface brightness (Bershady, 2000). For this reason, this parameter may potentially be an important marker of early stages of galactic mergers where tidal tails and disruptions form at larger scales.

Asymmetry Parameter

Asymmetry parameter A quantifies the degree of rotational symmetry of galaxy's light. It is determined by rotating individual galaxy's image around its center and subtracting flux values from its original image. That is, it compares fluxes on the opposite ends of galaxy. Asymmetry parameter A is defined as (Conselice, 1997):

$$A = \frac{\sum_{x,y} |I_{(x,y)} - I_{180(x,y)}|}{2\Sigma |I_{(x,y)}|} - B_{180}$$
(2)

where $I_{(x,y)}$ is the flux in pixel (x,y), $I_{180(x,y)}$ is the flux in pixel 180(x,y) in the image rotated about galaxy's central pixel by 180° and B₁₈₀ is the average background asymmetry. Lowest value of the asymmetry parameter is 0 while the highest is 1. Zero asymmetry would correspond to a completely symmetric galaxy (each point of light has a corresponding point of identical brightness at the same distance from the center, rotated by 180°) and A=1 would correspond to a totally asymmetric one (no point of light has a corresponding point of identical brightness).

For example, galaxies with smooth elliptical light profiles have high degree of rotational symmetry, while spiral galaxies are less symmetric, and irregular ones are highly asymmetric.

An attempt to examine asymmetry and its usefulness as a morphological parameter was made by Conselice 1997. Their results have shown that asymmetry grows with Hubble type (albeit with high dispersion) (Figure 2). A more useful correlation was noticed between color (spectral type) and asymmetry (Figure 2).

We expect the asymmetry parameter to be important marker of early and mid merger stages of galactic interactions where tidal disruptions are most prominent, though we expect that the sensitivity of this parameter will decrease with redshift due to decrease in resolution.



Figure 2. Left: The relationship between the morphology as given in the RC3 Catalog (de Vaucouleurs et al. 1991) and the symmetry numbers in the J band. The numbers represent the various morphological types (-1,0, 1, 2, 3, 4, 5, 6, 7 are E, SO, Sa, Sab, Sb, Sbc, Sc, Scd, Sd, respectively). Right: The symmetry-color relation. An excellent correlation is found between these two physical parameters suggesting that this relationship is a fundamental one to galaxies (Conselice, 1997)

Clumpiness Parameter

Unlike elliptical galaxies, which are generally very "smooth" objects, spiral galaxies (galaxies undergoing star formation) usually contain clumpy structures. In order to quantify this, a new parameter called clumpiness parameter S was defined as the ratio of the amount of light contained in clumpy structures to the total amount of light in the galaxy.

In order to compute the value of S, the galaxy image needs to be smoothed by a filter of width σ , and then subtracted by the original image. Using this method, a residual map that contains only the high-frequency components of the galaxy's stellar light distribution is produced.

Parameter is computed using the following equation (Conselice, 2003):

$$S = 10 \times \sum_{x,y=1,1}^{N,N} \frac{(I_{x,y} - I^{\sigma}_{x,y}) - B_{x,y}}{I_{x,y}}$$
(3)

where $I_{x,y}$ is sky-subtracted flux value of the galaxy at position (x, y), $I'_{x,y}$ is value of the galaxy's flux at (x, y) once it has been reduced in resolution, $B_{x,y}$ are background pixel values in an area of the sky which is equal to the galaxy's area and N is size of the galaxy in pixels.

For elliptical galaxies this ratio is generally near zero which is expected as they are quite smooth in structure and do not host star-forming regions. Furthermore, we expect this parameter to be a good indicator of late merger stages, where there is one galaxy which hosts two cores, just before the final coalescence. We note, however, that the sensitivity of this parameter will decrease with redshift and probably even more rapidly than asymmetry parameter, due to decreasing resolution.

Gini Coefficient

Originally used in econometrics for measuring the distribution of wealth within a population (Lorenz, 1905), Gini coefficient (G; Figure 3) was found useful in astrophysics for finding the quantitative measurement of inequality of galaxy light distribution between pixels (Lotz et al., 2004). That is, Gini coefficient tells us if there is a perfectly equal distibution of light among all pixels (if some fraction of all pixels contains that same fraction of total light), and if not, how far this distribution is from perfect.



Figure 3. The x-axis corresponds to the quantile of the distribution, and the y-axis corresponds to the cumulative proportion. Lorenz curve: the Gini coefficient is the area between the Lorenz curve of the galaxy's pixels and that of equitable distribution (shaded region). The given curve for galaxy of type S0 NGC 4526 has G = 0.59 (Lotz et al., 2004).

G is therefore calculated by applying the following equation to the list of pixel values sorted by intensity as:

$$G = \frac{1}{\bar{X}n(n-1)} \sum_{i=1}^{n} (2i - n - 1)Xi$$
(4)

where \overline{X} is the mean over all (pixel flux) values Xi, and n is number of pixels in a galaxy.

There is a strong correlation between Gini and asymmetry coefficients, (G and C), for local galaxies (Figure 4), which could be an indication that G is a suitable replacement for C measurements at high redshifts. Although G measurements do not depend on galaxy shape, using it as a direct substitution for C would be oversimplification, at least for nearby galaxies where the contribution of irregular galaxies is rather small (Abraham et al., 2003). Gini coefficient is a good marker of an overall smoothness and as such it can be a good probe of merger process in general. Thus, one of the key goals is to see how Gini coefficient evolves with merger process. Tracking its fractional evolution might remove dependance on galaxy type and provide a more objective measure of merger stage.



Figure 4. Gini coefficient vs. central concentration for the i-band sample (Abraham et al., 2003)

M₂₀ Parameter

The normalized second-order moment of the brightest 20% (M₂₀) of the galaxy's flux has proven itself useful for tracing the spatial distribution of structures such as bars, spiral arms, bright nuclei and off-center star clusters. M₂₀ is computed by ordering galaxy's pixels by flux, summing M_i (flux in each pixel multiplied by the squared distance to the center of the galaxy) over the brightest pixels until the sum of the brightest pixels equals 20% of the total flux, and then normalizing by M_{tot} .

$$M_{20} \equiv \log_{10} \left(\frac{\Sigma_i M_i}{M_{tot}} \right), \qquad while \ \Sigma_i f_i < 0.2 f_{tot}$$
(5)

Here f_{tot} is the total flux and f_i are fluxes for each pixel. M_{tot} is the flux in each pixel multiplied by the squared distance to the galactic center, summed

over all pixels, and the normalization by M_{tot} removes the dependence on total galaxy flux. M_{20} seems to be more sensitive than concentration C to merger signatures such as multiple nuclei which form at later merger stages, since it is not measured within circular or elliptical apertures and also because the galactic center is a free parameter (Lotz et al., 2004). This parameter may be found useful as a marker of late merger stages where galaxies enter star-burst phase and prominent regions start showing up in brightness. This will hopefully help us differentiate between star-forming and non-star-forming processes (tidal shocks and active galactic nuclei).

DISCUSSION

Identification of galaxy types relies on successful and detailed descriptions of their morphology. Different methods for parametrising and quantising galactic morphology have been developed and parameters identified. However, when observing systems at large redshifts not all parameters are as easily accessible and estimated. Furthermore, not all parameters are equally reliable indicators of galactic history. The purpose of this overview article was to list all morphology parameters in use and disscuss their benefits and weaknesses with respect to applying them at high-redhisft systems.

CONCLUSION

All of the mentioned parameters provide new possibilities for determining morphology of galaxies, especially irregular ones. Gini coefficient G and M_{20} parameter in particular could give us a new insight into galaxy merger morphology and its evolution according to Lotz et al. (2008a and 2008b). Our future goal is to explore these parameters on local merging systems and to identify which parameter is most sensitive to what merger stage. Furthermore, in the future we will investigate whether there is a correlation between G and M_{20} and merger stage (according to Haan et al. 2011, classification scheme), where the final goal would be to obtain a better insight into changes of FIR-radio correlation in interacting galaxies (Pavlovic & Prodanovic, 2019).

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ИСПИТИВАЊЕ МОРФОЛОГИЈЕ ГАЛАКСИЈА НА ВЕЛИКИМ ЦРВЕНИМ ПОМАЦИМА

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РЕЗИМЕ: Опште је познато да су неправилне галаксије последица судара или блиских пролаза међу галаксијама. Код нама блиских галаксија (у локалном универзуму) одређивање морфолошког типа и стадијума судара не представља велики проблем. Међутим, када су у питању галаксије које се налазе на великим црвеним помацима, одређивање морфологије није тривијалан проблем. У овом раду биће детаљно описани морфолошки параметри који се користе за одређивање морфологије галаксија на великим црвеним помацима. Циљ овог истраживања јесте да се морфолошки параметри, у будућем раду, испитају у односу на стадијум судара код галаксија у Локалном универзуму, те да се на тај начин можда пронађе јединствени параметар који се може применити на проучавање стадијума судара далеких галаксија.

КЉУЧНЕ РЕЧИ: велики црвени помаци, морфологија галаксија, морфолошки параметри

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MERCURY CONTENT AND DISTRIBUTION IN HOUSEHOLD DUST AND SOIL IN THE TOWN OF ŠID

ABSTRACT: Mercury (Hg) and potentially toxic elements (PTEs) are components of household dust and are a risk for human health. The aim of this study was to determine the concentrations of Hg and PTEs in household dust in individual housing facilities in the town of Šid, Serbia and their correlation to the content of the elements found in the surrounding garden soil. Total of 64 samples of household dust were collected from 16 locations of individual housing facilities on 4 occasions. Samples of surrounding yard and garden soil were simultaneously collected. None of the 64 analysed soil samples exceeded the threshold limit (TL) prescribed by law for non-agricultural soil, which is 0.3 mg kg⁻¹ DM. Content of Hg in household dust was much higher than in the surrounding soil and ranged from 0.005 to 1.566 mg kg⁻¹ DM. The resulting values of PTEs (As, B, Co, Cr, Cu, Ni, Pb, and Zn) in household dust had a significantly higher range than in the soil. Contents of Hg, B, Cu, and Zn were much higher in household dust than in the soil. According to the results of correlations of the analysed elements in household dust and surrounding soil, and according to the analysed locations, direct effect of Hg and PTEs contents on the content of the same elements in household dust was not confirmed.

KEYWORDS: mercury, potentially toxic elements PTEs, household dust, soil, Šid

INTRODUCTION

Mercury (Hg) is the only metal that easily changes state of aggregation, which allows it to migrate throughout the environment and enter organic and inorganic compounds with various levels of toxicity. There are studies on

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harmful materials in household dust which indicate health issues related to the dust (Casley et al., 2018), and studies on mercury risk assessment and bioavailability (Rasmussen et al., 2008; 2011; 2013; MacLean et al., 2011; Cukrowska, 2018). However, there are scarce studies on the origins of such contamination. It is assumed that Hg comes from different household appliances, from air (from coal burning products in rural areas), or indirectly from the atmospheric accumulation in the surrounding soil and consequent transfer into household dust. According to some studies, Hg in household dust may also originate from the construction materials of the facilities (Hagan et al., 2013).

The Minamata Convention on Mercury was approved and signed in Geneva in 2013 and entered into force in 2017. It is an international treaty designed to protect human health and the environment from the harmful effects of mercury. Control and monitoring of anthropogenically introduced Hg throughout its life cycle is the key factor in fulfilling the obligations set by the Convention, and the Republic of Serbia is a signatory country to the treaty. Sources of Hg are mostly historical pollution from chlorine production, Hg fluorescent lamps, gold and silver extraction and processing, application of dental Hg amalgam and instruments for measuring various physical quantities (thermometers, barometers). Other sources may include formerly used wood preservatives, seed protection chemicals, pesticide and paint industry, etc. (Adamov, 1984).

Potentially toxic elements (PTEs) are a group of elements in the environment which could be harmful to biota if found in higher concentrations. Some of these elements present in the soil (such as Co, Zn, Cu, B, etc.) simultaneously serve as nutrients to higher plants if present in optimum concentrations. PTEs in the soil can be of natural, geochemical origin, from parent material or from anthropogenous sources. PTEs reach the soil and household dust mostly by atmospheric deposition from various sources. In an indoor atmosphere these substances become deposited on different surfaces along with other components such as mold spores, mites and their excrement or discharge (Wisniewska et al., 2017). Multi-element analyses of household dust differ significantly from multi-element analyses of soil and street dust. The data show that higher concentrations are found in household dust samples from older homes (Rasmussen et al., 2001).

The aim of this study was to determine Hg and PTE concentrations in household dust in individual housing facilities in the town of Šid and their correlation to the content of the same elements found in the surrounding garden soil.

MATERIAL AND METHODS

Sample collection and processing

Samples of soil and household dust were collected simultaneously from 16 locations of individual housing facilities in the town of Šid, Serbia (45.1224° N, 19.2209° E) on four occasions: 21 September 2016, 3 December 2016, 5 March 2017 and 14 May 2017. Total of 64 samples of soil and household dust were col-

lected in this way. Soil samples were taken from yards and gardens from 0-20 cm depth, so that one composite sample is a collection of several individual samples. Household dust was collected indoors by vacuuming recent surface dust into clean sample bags. Larger solid particulates were removed from these dust samples (circa 100 g); the samples were then sieved and dried at room temperature.

Laboratory analyses

Laboratory analyses were performed at the Laboratory of the Faculty of Environment Protection, University Educons in Sremska Kamenica, Serbia and Laboratory for Soil and Agroecology of the Institute of Field and Vegetable Crops in Novi Sad, Serbia.

The soil samples were air-dried at the room temperature, milled and sieved to <2 mm particle size, in accordance with ISO 11464 (2006).

The samples were analysed for total Hg content using Direct Mercury Analyzer DMA 80 Milestone, which combines techniques of thermal decomposition, catalytic conversion, amalgamation, and atomic absorption spectrophotometry (λ =253.65 nm) in solid samples. Quality assurance and quality control (QA/QC) were conducted by certified reference material BCR 142R. The accuracy was within interval 92.84–109.70% and recovery was 101.11%.

The samples were analysed for pseudo-total contents of PETs (As, B, Co, Cr, Cu, Ni, Pb, Zn) after wet digesting the soil and household dust in concentrated HNO₃ and H_2O_2 (5HNO₃: $1H_2O_2$, and 1:12 solid-to-solution ratio). The concentration of elements was determined by ICP-OES (Vista Pro-Axial, Varian) in accordance with US EPA method 200.7:2001. Quality control was periodically carried out with reference materials ERM CC 141 (contains all examined elements, except B) and deviations were within $\pm 10\%$ of the certified values.

Statistical analysis

Data were statistically processed by analysis of the main descriptive parameters for each element. Statistical parameters were shown in tables and box-plots graphs. The significance of differences in measured parameters between the elements was determined using Fisher's LSD test ($p \le 0.05$). All statistical analyses were performed using STATISTICA for Windows version 12 (Dell Inc. 2016).

RESULTS AND DISCUSSION

Concentration of Hg and PTEs in soil and dust

Significant correlation was not found between 4 occasions of sampling Hg and PTEs in household dust and surrounding soil on the analysed 16 locations. Regarding soil samples, this may be explained by too short a period between

sample collection occasions (12 days, 90 days and 70 days) to expect any increase in contents of the analysed elements in the soil.

Regarding Hg content in soil, none of the 64 analysed samples exceeded the threshold limit (TL) for soil, which is 0.3 mg kg⁻¹ according to the Decree on Limit Values for Polluting, Harmful and Hazardous Substances in the Soil (*Official Gazette RS*, 30/2018) for non-agricultural soil. Hg content in household dust was much higher than in the surrounding soil and ranged from 0.005 to 1.566 with a mean value of 0.126 mg kg⁻¹ (Table 1). The average Hg value of present research is lower compared to the obtained average value of the city of Novi Sad 0.32 (Kastori et al., 2009), and of the city of Budapest 1.03 mg kg⁻¹ (Kastori et al., 2011) implying urban population size factors. Hg concentration in percentile interval 25–75% ranged from 0.061 to 0.328 mg kg⁻¹ and was higher than the same interval for the soil (Figure 1). The concentration of toxic metals in household dust may be from 2 to 32 times higher than levels found in garden soil around the house (Rasmussen et al., 2001). It is hard to explain the origin of Hg in indoor dust samples. One of the possibilities of Hg source might be the damaged formerly used Hg thermometers (Kastori et al., 2009).

According to the defined ranges of concentrations for urban dust in Shanghai city (Wang et al., 2009), this study found 70% of household dust to be of clean level concentration ($<0.259 \text{ mg kg}^{-1}$), 9% of the samples were slightly polluted ($0.259-0.518 \text{ mg kg}^{-1}$), as much as 17% of samples were moderately polluted ($0.518-1.295 \text{ mg kg}^{-1}$) and 3% of samples were heavily polluted ($>1.295 \text{ mg kg}^{-1}$).



Box Plot of Hg dust

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Figure 1. Box-plots graphic display of Hg in household dust and soil (mg kg⁻¹ DM)

Some studies on the presence of mercury in household dust (Rasmussen et al. 2001) found total Hg concentration to range from 1.6 to 12.6 mg kg⁻¹ in 40 dust samples. The highest Hg concentrations were reported in former Hg mining districts. In the Idrijan urban area (Slovenia), aqua regia soluble Hg (ARS Hg) was reported in topsoil in concentration of 8 to 1.210 mg kg⁻¹ and in household dust, with 6 to 120 mg kg⁻¹ (Bavec et al., 2018). Total Hg concentrations ranged from 3.06 to 926 mg kg⁻¹ in dirt floors in Huancavelica, Peru (Hagan et al., 2013).

According to the box plots for other PTEs (figures not shown), as well as for Hg content (Figure 1), the obtained PTE values in household dust had statistically significant higher range with a larger number of extremes and outliers, which was expected when compared to the soil.

Contents of Hg, B, Cu, and Zn were much higher in household dust than in the surrounding soil. Pb content was higher in the dust, while Ni and Co contents were relatively higher in the soil. Contents of As and Cr were relatively even in the household dust and in the surrounding soil (Table 1). Relatively high concentrations of Cu and Zn found in this study are in accordance with other studies on office and household dust (Kefeni and Okonkwo, 2013) and street dust (Kastori et al., 2010; Žibret et al., 2013). Results of many researches suggest that there are a huge number of indoor pollutants sources such as: indoor activities, emission from building materials and furnishings, indoors tobacco smoking, heating, pets, cooking activities etc. Accordingly, pollutants can originate from indoor environment itself as well as from outdoor source (Kastori et al., 2011).

ma ka -1		Home dust			Soil	
iiig kg	min	max	mean	min	max	mean
Hg	0.005	1.566	0.126	0.00005	0.244	0.0257
As	2.4	12.9	5.0	3.3	10.5	6.8
В	6.9	221.1	28.3	2.3	10.4	5.5
Co	2.1	10.5	3.4	4.7	10.2	7.8
Cr	4.5	83.2	24.7	20.1	41.6	27.6
Cu	5.6	883.0	55.0	16.8	66.6	30.4
Ni	5.0	80.4	20.8	17.2	40.5	24.1
Pb	11.3	112.6	24.7	9.6	36.6	17.2
Zn	62.7	893.5	365.3	57.3	262.0	110.2

Table 1. Minimum, maximum and mean value of PTEs in household dust and soil (mg kg⁻¹ DM)

Correlations of Hg and PTEs between soil and dust

Hg in household dust was significantly positively correlated with B and Pb, and negatively correlated with Zn (Table 2). Soil Hg was not significantly correlated with either of the PTEs (Table 3). The obtained results show high Hg mobility throughout the environment, so this element does not correlate with other PTEs deposited into soil by atmospheric deposition.

There was a larger number of significant correlations between PTEs in the soil than in the household dust (Tables 2 and 3). In household dust, there were negative correlations between Cr and As, and between Zn and As (Table 2), while the same elements were positively correlated in the soil (Table 2). On the other hand, the following elements were positively correlated both in the dust and in the soil: Ni and Cr; Pb and Co; Zn and Cr, Cu and Ni. The results show heterogeneity of the potential PTE sources in household dust.

	As	В	Со	Cr	Cu	Ni	Pb	Zn
Hg	0.1803	0.3351*	0.1771	-0.1343	-0.1782	-0.0973	0.2606*	-0.2526*
As		-0.1416	0.2255	-0.2723*	-0.1337	-0.2373	0.1048	-0.3580*
В			-0.0586	0.0785	0.1200	0.1914	0.2165	0.2000
Co				-0.2263	-0.0650	-0.2160	0.2705	-0.2354
Cr					0.2301	0.5746*	0.0496	0.3504*
Cu						0.1879	0.2113	0.6090*
Ni							0.0484	0.4218*
Pb								0.2021

Table 2. Correlations between PTEs in household dust for all locations

* p≤0.05, significantly correlated

	As	В	Со	Cr	Cu	Ni	Pb	Zn
Hg	-0.0996	0.2078	-0.1526	-0.0697	0.1342	0.0081	-0.0041	0.1059
As		0.4310*	0.3672*	0.6464*	0.2787*	0.5795*	0.4464*	0.4974*
В			0.2388	0.4175*	0.4175*	0.2374	0.3046*	0.2976*
Co				0.6176*	0.3073*	0.3955*	0.5158*	0.2978*
Cr					0.2993*	0.8711*	0.6619*	0.5928*
Cu						0.2711*	0.5019*	0.4539*
Ni							0.6229*	0.6566*
Pb								0.7166*

Table 3. Correlations between PTEs in soil for all locations

* p≤0.05, significantly correlated

In order to determine the effects of PTE concentration in household dust, the correlations between Hg and PTE concentration were separately statistically processed for each of the analysed locations (data not shown). In 1,024 pairs (16 locations and 8 elements), significant correlations were found in 85 cases. Out of those, 12 cases had the same element positively correlated between dust and soil. According to the elements, Hg was positively correlated between dust and soil on two locations. Pb, Ni and Cu were positively correlated between dust and soil on two locations, and Zn and Cr on one location. The obtained results show that Hg and PTEs from the surrounding soil of yard and garden can affect their concentration in the indoor household dust; however, this has not been widely confirmed in this study. The results are in accordance with Rasmussen et al. (2001) who concluded that metal concentration in indoor dust cannot be predicted from outdoor soil levels.

CONCLUSION

Statistically significant correlation between the four sampling occasions on the analysed 16 locations was not found.

None of the 64 analysed samples exceeded the threshold limit (TL) for soil, which is 0.3 mg kg⁻¹. Content of Hg in household dust was much higher than in the surrounding soil and ranged from 0.005 to 1.566, with a mean value of 0.126 mg kg⁻¹.

Measured values of PTEs (As, B, Co, Cr, Cu, Ni, Pb, and Zn) in household dust had significant higher range than in the surrounding soil. Contents of Hg, B, Cu and Zn were much higher in household dust, content of Pb was higher in dust and contents of Ni and Co were relatively higher in soil. The contents of As and Cr were relatively even in dust and in soil. Relatively high concentrations of Cu and Zn found in this study are in accordance with other studies.

According to the results of correlations of the analysed elements in household dust and surrounding soil, and according to the analysed locations, direct effect of Hg and PTEs contents on the content of the same elements in household dust was not confirmed.

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САДРЖАЈ И ДИСТРИБУЦИЈА ЖИВЕ (Hg) У КУЋНОЈ ПРАШИНИ И ЗЕМЉИШТУ ГРАДА ШИДА

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РЕЗИМЕ: Жива и потенцијално токсични елементи (ПТЕ) као саставни део кућне прашине представљају ризик за људско здравље. Циљ овог рада је утврђивање концентрација Нд и ПТЕ у кућној прашини, у објектима индивидуалног становања, града Шида и њихова веза са садржајем елемената у околном земљишту башта и окућница. Узето је 64 узорка кућне прашине на 16 локација индивидуалног становања у четири временска периода. Истовремено су узети узорци околног земљишта окућница и башта. Ниједан од 64 испитивана узорка земљишта не прелази законски прописану граничну вредност (ГВ) за непољопривредно земљиште од $0,3 \text{ mg kg}^{-1} \text{ CM}$. Садржај Hg у кућној прашини је много виши од околног земљишта и кретао се у интервалу од 0,005 до 1,566 mg kg⁻¹ СМ. Добијене вредности ПТЕ (As, B, Co, Cr, Cu, Ni, Pb, Zn) у кућној прашини имају статистички значајан већи распон у односу на земљиште. Садржај Hg, B, Cu, Zn је много већи у кућној прашини у односу на околно земљиште. Према добијеним корелацијама посматраних елемената у прашини и земљишту, као и према посматраним локацијама, генерално није утврђен директни утицај садржаја Не и ПТЕ у земљишту на садржај ових елемената у кућној прашини.

КЉУЧНЕ РЕЧИ: жива (Hg), потенцијално токсични елементи ПТЕ, кућна прашина, земљиште, град Шид

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GUT MICRO FLORA IN GENESIS OF METHILTHYO METABOLITES OF PARACETAMOL

ABSTRACT: Gut micro flora represents a system of extensive metabolic capacity which is quite different when compared to other cells of body and organs. In recent times, there has been an evidence of very important role of gut cysteine conjugate beta-lyases in metabolism of cystein conjugates. A lot of studies are dedicated to exploration of the role of gut micro flora in formation of methylthio adducts from paracetamol in conventional mice and those treated with neomycin as our study. A highly significant reduction in urinary excretion of 3-methylthioparacetamol in group of neomycin-treated mice was confirmed as well its glucuronic acid and sulphate conjugates. Following the principal role of gut flora in the C-S cleavage of paracetamol I-3-cyctein it is clear that highest concentration of methylthio adducts from paracetamol are presented in pretreated mice.

KEYWORDS: gut micro flora, metabolism, paracetamol

INTRODUCTION

The study of the role of intestinal flora in the metabolism of numerous xenobiotics is the subject of numerous studies (Bojic et al., 2014, 2015; Smieško et al., 2018a,b). In recent years, the interest in researching the role of the beta-lyase enzyme produced by the intestinal flora has been particularly discernible. Beta lyase transformed conjugates of cysteine originating from xenobiotics of the toxic metabolites (Mikov, 1994; Li et al., 2016). These toxic products are thiols or other metabolites derived from thiol. The intestinal flora may, by the action of its enzymes, split the conjugate glutathione into the corresponding S-substituents of cysteine (Westman et al., 2012). Although the first data on the formation of thiols from the cysteine congenital cysteine is 50 years old (Di, 2014), it has been

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worth to note the importance of these pathways that lead to the formation of various thiomethyl conjugates from numerous xenobiotics.

A significant portion of the widely used paracetamol analgesic is metabolized by pathways that are not dependent on cytochrome P-450, which leads to its conjugation with glutathione (Cho and Blaser, 2012). This conjugate is mostly excreted in the urine as cysteine, mercapturate, and methylthio derivatives (Li and Jia, 2013; Davidson and Eastham, 1966). In all this, intestinal flora has the role of the krill (Smith and Macfarlane, 1997; Larson et al., 2005).

The aim of this paper was to experimentally examine the importance of gastrointestinal flora in the formation of methylthio metabolite paracetamol.

MATERIAL AND METHODS

Neomycin sulphate and paracetamol are purchased from Sigma Chemical Company, United Kingdom. Metabolites of paracetamol are a gift from Sterling-Winthrop.

Two groups of 8 pairs of white laboratory BALB / c mice (body weight 33 ± 1 g) received neomycin sulphate (500 mg/kg in saline) or saline (10 ml/kg) orally, twice a day for three days. On the fourth day, each animal administered paracetamol (200 mg/kg) was dissolved in saline solution in the form of intraperitoneal injection.

After injection of paracetamol, each mouse was placed in a separate metabolic cage, which allowed the extraction of urine and feces. Samples of eighthour urine were collected in dark-walled tubes and kept at 0 °C. After an eight-hour period, the cages were washed with 5 ml of water, and the wash was added to the urine and stored all the way to 20 °C until analyzed. Paracetamol and its metabolites were separated and quantitatively determined using HPLC.

The obtained results were expressed as the mean value of +/- SD, and the significance of the difference between the groups was determined using Student t-test.

RESULTS

By measuring the concentration of metabolites in cochlear urine, the main metabolites of paracetamol secreted in the urine were paracetamol-glucuronide, paracetamol-cysteinate and paracetamol sulphate, unchanged paracetamol, methylthioparacetamol as its glucuronide, sulphate and sulfoxide (Table 1). The total amount of depleted paracetamol in eight-hour urine remained unchanged after the third day of neomycin and the differences between the treated and control mice were mirrored in a highly significant reduction in the excretion of various 3-methylthio metabolites, or a significant reduction in paracetamol 3-methylthio metabolite in the neomycin of the treated group.

Metabolites	Control group (X+/- SD)	Neomicyn treated group (X+/-SD)
Paracetamol glucuronide	425.6+/-9.06	42.2+/-5.44
Paracetamol sulphate	7.3+/-1.62	7.8+/-1.4
Paracetamol cisteinate	15.17+/-3081	15.14+/-3.44
Unchanged	4.9+/-1.22	4.27+/-1.75
Mercapturate	0.34+/-0.510	0.107+/-0086
Thiomethyl sulphate and Methyl thiosulphoxide	2013+/-1.22	0.22+/-0.29*

Table 1. Excretion of metabolites of paracetamol in 8 hours urine presented as relative value (%) of total intraperitoneal ordinate paracetamol (N=8, p<0.001)

DISCUSSION

Paracetamol with metabolites is discussed in three basic ways (Cho and Blaser, 2012):

1) conjugated with glucuronic acid,

2) conjugation with sulfates and

3) metabolic activation of microsomal monooxygenase.

This last way creates a reactive intermediate product that is inactivated by conjugation with glutathione. This glutathione conjugate is largely transformed before elimination (Lee et al., 2012), and the current data indicate the contribution of intestinal micro flora in these processes, especially in the production of 3-methylthio-paracetamol. S-glutathosate is metabolized by hydrolysis in S-cysteinate, which has at least 3 different fates: N-acetylation (when mercapturic acid occurs), transamination and cleavage of C-S bond (Gamage et al., 2006).

This last time leads to the production of thiol. Tri-thioparacetamol is a highly reactive compound (18) that can contribute to the toxicity of the parent drug. The thiols are subjected to methylatio at once to form a methylthio product which can be transformed into S-oxides: sulfoxide and sulfone (Di, 2014). For now, a particular difficulty is the localization of the C-S lyase of cysteine conjugates in the tissue.

In animals treated with neomycin, there was a reduced excretion of thiomethyl metabolite. Although it is possible that neomycin modifies the reabsorption of glutathione metabolite of paracetamol secreted via the bile from the gut, these findings indicate the role of intestinal micro flora in the metabolism of paracetamol.

CONCLUSION

Gut micro flora is important for the formation of metabolic precursors of thiomethyl conjugate and its oxidation products. A particular importance ought to be paid to the consideration of the metabolic role of the intestinal micro flora from the toxicological aspect and its urine to paracetamol-3-cisetinate (Vick and Acheson, 1957; Stojančević et al., 2014).

Based on the results obtained, the intestinal micro flora plays an important role in the metabolism of paracetamol, which indicates the formation of methylthio metabolite paracetamol.

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ЦРЕВНА МИКРОФЛОРА У ГЕНЕЗИ МЕТИЛТИО МЕТАБОЛИТА ПАРАЦЕТАМОЛА

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САЖЕТАК: Цревна флора представља систем са обилним метаболичким капацитетом који је у потпуности другачији од других ћелија тела и органа. У новије време евидентирана је битна улога beta-lijaza коњугата цревног цистеина у метаболизму коњугата цистеина. Бројне студије су посвећене изучавању улоге цревне микрофлоре у стварању метилтио продуката из парацетамола код конвенционалних мишева и неомицином третираних као у овој студији. У групи третираних мишева неомицином запажена је високо значајна редукција урином излученог 3-metiltioparacetamola, као и присуство коњугата глукуронске киселине и сулфата. Узимајући у обзир основну улогу цревне флоре у деловању на C-S везе у парацетамола 1-3 цистеину, потпуно је јасно да су високе концентрације метилтио продуката парацетамола присутне код превише третираних мишева.

КЉУЧНЕ РЕЧИ: цревна микрофлора, метаболизам, парацетамол

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IDENTIFICATION AND PHYLOGENETIC ANALYSIS OF Fusarium proliferatum ISOLATED FROM ELEPHANT GARLIC Allium ampeloprasum L.

ABSTRACT: Symptoms of cloves rot of *A. ampeloprasum* were noticed during 2018 in storage conditions. 16 isolates were obtained (AMP1-AMP16) and according to morphological and cultural characteristics they belong to *F. proliferatum* (sex. stage *Gibberella intermedia*), species of *Gibberella fujikuroi* complex. To confirm morphological identification, total genomic DNA was extracted from mycelium of the 16 isolates by amplification of TEF-1a gene, using polymerase chain reaction (PCR) that was performed with the primer pair EF1 and EF2. Results presented in this article clearly indicated that the new host of *Fusarium proliferatum* as the causal agent of cloves rot is "elephant garlic" *Allium ampeloprasum*. Pathogenicity test was confirmed on *Allium ampeloprasum* cloves. Pathogenicity assays revealed that all isolates caused symptoms on tested *Allium* spp., like naturally infected cloves.

KEYWORDS: Allium ampeloprasum, Fusarium proliferatum, cloves rot

INTRODUCTION

Allium ampeloprasum (Family Amaryllidaceae Subfamily-Allioideae) is a medicinal plant well known for its pharmaceutical potential with characteristic large mature cloves, commonly known as "elephant garlic" (Sharifi-Rad et al., 2016). It is native in range from southern Europe to western Asia, but it is naturalized and cultivated worldwide. In Serbia, wild form of *A. ampeloprasum* is kept in the collections of the Institute of Field and Vegetable Crops (curator

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Jelica Gvozdanović-Varga) and it is used in the garlic (*Allium sativum* L.) breeding research program. Symptoms of cloves rot of *A. ampeloprasum* were noticed during 2018 in storage conditions. Most of the diseased cloves did not show visible symptoms, but after cleaning and when they were peeled, deep lesions covered with fungal growth were observed (Figure 1a, 1b). According to Fuentes et al. (2013), the frequent occurrence of bulb rot during storage period has become a limiting factor to garlic production, emphasized *F. proliferatum* as a major postharvest issue. The first report of *F. proliferatum* as a causal agent of garlic rot came from Germany (Seefelder et al., 2002), and subsequently it was reported in North America (Dugan et al., 2003), Serbia (Stanković et al., 2007), Spain (Palmero et al., 2008) and India (Sankar and Prasad Babu, 2012). Based on pathogenicity tests, Stanković et al. (2007) claimed that *F. proliferatum* should be regarded as a potentially serious pathogen of garlic in Serbia.



Figure 1. Fusarium proliferatum: a) and b) Symptoms on infected *Allium ampeloprasum* cloves – natural infection; c) Seven-day-old colony of isolate AMP11 grown on PDA – photographed from the top; d) Violet pigmentation released in PDA medium – bottom side of the agar plate.

MATERIAL AND METHODS

Isolation

In order to isolate the disease causing organism, cloves were separated, peeled off, surface disinfested in 1% NaOCl for 2 min, rinsed with sterilized distilled water, dried on a sterile filter paper and small parts of infected tissues were plated onto the medium surface. Potato dextrose agar (PDA) was used with the addition of 300 mg/l antibiotic (streptomycin sulfate). After 7 days at 25 °C, *Fusarium* colonies were examined and 16 isolates (AMP1-AMP16) were subcultured using a single spore technique. Growth rates are based on radial growth in a Petri dish. Plates were arranged in an incubator according to the experimental design and colony diameter measurements were taken at 3, 5 and 7 days after plating. Morphological and cultural characterization of isolates, cultured onto both PDA and Carnation leaf agar (CLA), was done according to Gerlach and Nirenberg (1982) and Leslie and Summerell (2006).

Pathogenicity test

Artificial inoculation of the five cloves of *Allium sativum* (autumn cv. Bosut, Ranko) and *Allium ampeloprasum* (cv. Biser), was done by using 7 day old mycelia from each isolate grown on PDA as an inoculum according to the method described by Palmero et al. (2012) and Dugan et al. (2007). Disinfection was done by dipping of cloves in 0.5% NaOCl for 60 seconds and rinsing in sterile water after which small pieces of inoculum were placed in a depth of 4 mm into the clove using a 1-mm diameter probe. Inoculated cloves were placed in aseptic sealed plastic boxes and incubated for three weeks at 25 °C, after which re-isolation of the fungi was done. For each isolate, another set of five cloves was inoculated with sterile PDA as negative control.

Molecular identification

In order to confirm morphological identification, sequencing of TEF-1a gene was performed using polymerase chain reaction (PCR) with the primer pair: EF1 and EF2 (O'Donnell et al., 1998). To obtain a DNA sequence, a total DNA of the 16 isolates and positive control FE-3 was extracted directly from the 7 day old mycelium (~ 100 mg wet weight), with a Dneasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Identification of isolate AMP11 was performed by sequencing the translation elongation factor TEF-1a gene. Purification and sequencing of the amplified fragments were performed in Company MACROGEN, Seoul, South Korea (http://dna. macrogen.com, Korea). Sequences were analysed in the program Finch TV Version 1.4.0., and filed in the GenBank database under the National Center for Biotechnology Information (NCBI).

Phylogenetic analysis

Phylogenetic tree on individual translation elongation factor TEF-1 α gene sequences was constructed using the Maximum Likelihood (ML) phylogeny with 1,000 bootstrap repeats and pairwise deletion, implemented in MEGA 6 software (Tamura et al., 2013). Manual comparisons, corrections and alignments of the sequences were performed using CLUSTALW integrated into MEGA6 software. Kimura 2-parameter model, Gamma distributed (G) determined by Model test implemented in MEGA6 was used as the best fitting model of nucleotide substitution.

RESULTS AND DISCUSSION

Colonies of all investigated isolates were fast-growing, reaching 7–8.5 cm in diameter after 7 days, forming abundant, aerial white mycelium with violet to dark pigments released in PDA. According to Leslie and Summerell (2006), violet pigments are usually produced in the agar, but with overall pigmentation varying in intensity from nearly colorless to almost black. All isolates formed typical slightly curved macroconidia, with distinct foot cell, mostly three to five septate, measuring 32–55 μ m x 3.4–4.2 μ m. On CLA, one-celled, slightly pyriform microconidia were formed in long chains or coherent in the false heads. Chlamydospores were absent. According to morphological and cultural characteristics, all investigated isolates belong to *F. proliferatum* (sex. stage *Gibberella intermedia*), species of *Gibberella fujikuroi* complex, Liseo-la section. Results presented in this article are in compliance with species descriptions given by Gerlach and Nirenberg (1982) and Leslie and Summerell (2006).

Pathogenicity test was performed on *Allium sativum* (autumn cv. Bosut, Ranko) and *Allium ampeloprasum* (cv. Biser). Pathogenicity assays revealed that all tested isolates caused symptoms on tested *Allium* spp., like naturally infected cloves (Figure 2). Regarding the daily mycelial growth rate isolates AMP7, AMP11, and AMP13 were the fastest-growing and they caused rotting cloves after 7, 13 and 15 days, respectively. All isolates were re-isolated from symptomatic tissue thus fulfilling Koch's postulates. They were cryopreserved at -80 °C. No fungi recovered from control cloves.

As a result of molecular identification, the sequence of isolate AMP11 was deposited in the GenBank under Accession number MK061545. Genome sequence of TEF-1 α gene is considered as highly significant information on species level for the entire *Fusarium* genus (Summerell et al., 2003; Geiser et al., 2004). This study based on analysis TEF gene sequences confirmed that strain originated from *A. ampeloprasum* had 100% homology to sequences of *F. proliferatum* strains obtained from NCBI database.



Figure 2. Pathogenicity test performed on *Allium ampeloprasum*: Lesions on cloves covered with fungal growth of *F. proliferatum*

For better understanding of phylogenetic relationship of *F. proliferatum* isolate originated from elephant garlic, sequences were compared with sequences data set of *F. proliferatum* originated from garlic and *Fusarium* strains from different hosts. The sequence analysis of translation elongation factor EF-1 α gene, grouped in the same cluster consisted of *F. proliferatum* isolates from garlic: strains F1119, F1131 from Italy, A6m1 from Spain, B3 from Serbia, but also with strains CBS 131570 from wheat from Iran, G16NH2-2-27S-1 from China and F90 from strawberry originated from Spain (Figure 3). Strain M14022 originated from grape in China was clustered separately.



Figure 3. Maximum Likelihood phylogenetic tree of *F. proliferatum* isolates derived from a translation elongation factor EF-1 α gene. Bar – estimated nucleotide substitutions per site is 0.2.

Results presented in this article clearly indicated that *Fusarium proliferatum* is the causal agent of cloves rots of *Allium ampeloprasum*. This species, although widespread as a pathogen of sorghum, maize, asparagus, onion, and garlic has not been described as a pathogen of *Allium ampeloprasum*.

CONCLUSION

Garlic bulbs are routinely stored at room temperature for several months or in refrigerated chambers with a high percent of moisture. The presence of *Fusarium proliferatum* cause rot of bulbs and cloves and it can be a threat to the collection of various Allium species preserved in storages. It can increase rot progression and severity with impact on reduction in germination. To our knowledge *Allium ampeloprasum* in the new host of *Fusarium proliferatum* as causal of cloves and bulbs rot.

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ИДЕНТИФИКАЦИЈА И ФИЛОГЕНЕТСКА АНАЛИЗА Fusarium proliferatum ПРОУЗРОКОВАЧА ТРУЛЕЖИ ЛУКА Allium ampeloprasum L.

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РЕЗИМЕ: Симптоми трулежи ускладиштених ченова белог лука на врсти познатој као "elephant garlic" (*A. ampeloprasum*) запажени су током 2018. године. Прикупљен је велики број заражених узорака и након изолације одабрано је 16 изолата (AMP1-AMP16) за даља истраживања. На основу морфолошких и одгајивачких карактеристика је установљено да је проузроковач трулежи ченова гљива *F. proliferatum* (телеморф *Gibberella intermedia*), која припада комплексу *Gibberella fujikuroi*. Проучавањем патогености сви изолати су проузроковали симптоме трулежи на различитим врстама рода *Allium* spp. који су идентични природној инфекцији. У циљу потврде морфолошких одлика извршена је молекуларна идентификација методом ланчане реакције полимеразе (PCR) коришћењем пара прајмера EF1 и EF2 који амплификују TEF-1α ген, секвенционирање ДНК и филогенетска анализа добијених секвенци. Добијени резултати потврдили су да је *Fusarium proliferatum* проузроковач трулежи на врсти *А. ampeloprasum* као новом домаћину.

КЉУЧНЕ РЕЧИ: Allium ampeloprasum, Fusarium proliferatum, ченови белог лука

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INFLUENCE OF SALINITY ON THE GROWTH AND DEVELOPMENT OF PANSIES (*Viola x wittrockiana* Gams.)

ABSTRACT: The purpose of this paper is to determinate the influence of salinity on the growth and development of pansies (*Viola x wittrockiana* Gams.). The objective of the present study was to examine the cultivation area selection and use of pansies related to the autumn and winter flowering aspects. A total of 40 pansy seedlings were included in the study and allocated to four groups of ten each. The experiment conducted four involved treatments: control (K), treatment with 3g/l NaCl (T1), treatment with 5g/l NaCl (T2), and treatment with 7 g/l NaCl (T3). Concentrated water was used for watering the seedlings observed during growth. Over the course of the eight-week experiment, the following quantitative and qualitative properties of the pansy seedlings were measured: height, number of leaves, number of flowers, diameter of flowers, length of leaves, width of leaves, as well as the vitality and decorativeness of the pansy seedlings. The results obtained show that pansies can tolerate increased amounts of salt (5 g/l and 7 g/l NaCl) with adverse effects on the growth, development, vitality and decorativeness of the plant.

KEYWORDS: decorativeness, pansy, salinity, sodium-chloride (NaCl), treatments, vitality

INTRODUCTION

The global pollution of the pedosphere is one of the major challenges facing agriculture in the modern world. Furthermore, the salt contamination of urban soils, mostly due to road salting in the winter, also poses a pressing problem worldwide (Anastasijević, 2007). As a method of deicing, the roads in Serbia are sprayed with sodium chloride (NaCl) in the winter, which is an ionic compound widely applied for its low market value and great accessibility. However, sodium chloride exerts a negative effect on the ground and plant cover.

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According to Kelting et al. (2010), the greatest salt contamination magnitude and concomitant plant damage were determined on the downwind side of the road, depending on the road slope and drainage pattern. High soil concentrations of salt lead to higher ion concentrations in the soil. Consequently, the uptake of nutrients and water by the plant is seriously hindered, resulting in the reduction of plant growth. Flowering or ornamental plants are most adversely affected by high salt and ion concentrations in the soil as they have a very shallow root system, as well as gentle stems and leaves, and thus can uptake larger amounts of sodium chloride. According to Savé (2009), plants considered ornamental embrace all the species that provide aesthetic pleasure and improve both the environment and the quality of people's lives. The optimal concentration of sodium chloride in the soil solution is 0.001–0.01%. High salt concentrations not only exert harmful effects on plants, but also increase the soil pH values. They can also cause the deterioration of the soil structure, resulting in a difficult flow of air and water, which are vital to the biological processes occurring in the root system of the plant. Salinization has different effects on the physiology of plants: increased rates of respiration, ion toxicity, changes in the plant growth, decreased photosynthesis, and other changes which are due to the replacement of calcium with sodium (Khalid et al., 2010). The accumulation of salts in the plant's organs leads to the premature aging of the plant. Accordingly, the supply of plants with nutrients is reduced, and dark necrotic lessions appear first at the edges of the leaf, spreading gradually to the inside of the leaf. When the plant absorbs large amounts of salt, its photosynthetic capacity begins to decrease, ultimately resulting in leaf withering. Cassaniti et al. (2009) showed that the decrease in leaf area was the first visible effect of salinity in both sensitive and tolerant species such as Cotoneaster lacteus W.W.Sm and Eugenia myrtifolia Gaertn.

The purpose of this study is to determine the effect of salinity on the growth and development of pansies (*Viola x wittrockiana* Gams.) and to establish whether pansies are suitable for use in salt-affected flower beds.

MATERIALS AND METHODS

The pansy (*Viola x wittrockiana* Gams.) is an ornamental, flowering plant. It is quite durable, featuring flowers blooming throughout the season. The cool days of spring and autumn favor its growth, but it grows and blooms in the winter as well. The pansy (*Viola x wittrockiana* Gams.) has a very complex hybrid character. Three species from genus *Violaceae: Viola tricolor* L., *Viola altaica* Gawl. and *Viola lutea* Huds. were involved in the nascency of species *Viola x wittrockiana* Gams. Breeders have chosen these three species due to unusual combinations of colors and large flowers. The pansy is very suitable for the cultivation in parks, gardens and other green areas.

The present experiment was conducted in the Botanical Garden of the Faculty of Agriculture in Novi Sad. A total of 40 pansy seedlings were included in this study and allocated to four groups of ten each for four different treatments (Figure 1). The experiment took place over the period of eight weeks, and the seedlings were watered weekly. The salt treatments applied three NaCl concentrations involved and were labeled in the following manner: T1, T2 and T3. With the exception of the control treatment (K), the treatments T1, T2 and T3 entailed watering the pansy seedlings with a certain amount of salt, i.e. sodium chloride dissolved in water: T1 (3 g/l NaCl), T2 (5 g/l NaCl), and T3 (7 g/l NaCl). Prior to the experiment, all the seedlings were meticulously examined, especially the root system, and were found to have a healthy and well-developed root system without any damage. The pansy seedlings selected for the experiment period in order to facilitate their acclimatization and protection from cold and adverse weather conditions.



Figure 1. Setting up an experiment

The following parameters were measured throughout the experiment: height, number of leaves, number of flowers, diameter of flowers, length of leaves, width of leaves, as well as the vitality and decorativeness of the pansy seedlings.

The data collected were analyzed using the Statistica 12 (StatSoft, DELL) software, including basic statistical parameters such as the mean value and the Duncan's multiple range tests.

RESULTS

The morphological parameters examined in this paper are shown in Table 1. The experiment was conducted over the period of eight weeks. Table 1 displays the results obtained in the first, fourth and eighth experimental weeks.

First week							
	Height (cm)	Number of leaves	Number of flowers	Diameter of flowers (cm)	Lenght of leaves (cm)	Width of leaves (cm)	Vitality and deco- rativenes
K	13 ^{b*}	21 ^a	3 ^a	7.1 ^b	5.1ª	2.9 ^a	5 ^a
T1	13.1 ^b	19 ^a	2 ^b	8.3ª	5 ^a	3 ^a	4 ^b
Т2	13.5 ^b	18 ^b	2 ^b	7 ^b	5.2 ^a	3.1 ^a	4 ^b
Т3	14.3 ^a	20^{a}	2 ^b	8.2 ^a	5.1ª	3 ^a	4 ^b
Mean	13.47	19.50	2.25	7.65	5.10	3.00	4.25
			Fou	rth week			
	Height (cm)	Number of leaves	Number of flowers	Diameter of flowers (cm)	Lenght of leaves (cm)	Width of leaves (cm)	Vitality and deco- rativenes
K	13.3 ^b	23 ^b	3 ^a	8.1 ^a	5.2 ^a	3 ^a	5 ^a
T1	13.2 ^b	29 ^a	1 ^b	8.4 ^a	5.1 ^a	3.1ª	4 ^b
Т2	13 ^b	27 ^a	3 ^a	8 ^b	5.3 ^a	3.1 ^a	4 ^b
Т3	14.5 ^a	25 ^a	2 ^b	8.5 ^a	5.2 ^a	3 ^a	4 ^b
Mean	13.50	26.00	2.00	8.25	5.20	3.05	4.25
			Eigl	nth week			
	Height (cm)	Number of leaves	Number of flowers	Diameter of flowers (cm)	Lenght of leaves (cm)	Width of leaves (cm)	Vitality and deco- rativenes
K	13.3 ^b	20 ^b	3 ^a	8.2 ^a	4.2 ^b	3.1 ^b	4 ^a
T1	13.2 ^b	21 ^a	2 ^b	7.3 ^b	4.2 ^b	3 ^b	3 ^b
Т2	13 ^b	19 ^b	3 ^a	6.2 ^c	4.3 ^b	3.3 ^a	3 ^b
Т3	14.5 ^a	17 ^b	2 ^b	6 ^c	4.3 ^a	2.7 ^b	2 ^c
Mean	13.50	19.25	2.25	6.92	4.25	3.03	3.00

Table 1. Morphological parameters of the pansy seedlings examined

*Values marked with different letters differ significantly according to the Duncan's test of multiple intervals at p < 0.05.

The experiment was conducted over the period of eight weeks, and the pansy seedlings were watered weekly. During the first experimental week, minor changes in the leaves were determined: mild depressions, gentle curling and brown spots of the leaves due to the influence of salt. In the T1 treatment with 3 g/l NaCl, the leaves started losing their glow and became mildly rough. As for the plants treated with 5 g/l NaCl in the T2 treatment, the leaves exhibited mild depressions, accompanied by the yellowing and curling of the leaf surface. No significant changes were recorded in the plants treated with 7 g/l NaCl, as well as in the control plants. However, the plants treated with 7 g/l NaCl were found to exhibit notable changes in the fourth experimental week with high or invariant values of the parameters examined (namely height, number of leaves, as well as the vitality and decorativeness of the pansy seedlings) compared to those recorded in the first experimental week. In contrast, the plants treated

with 3 and 5 g/l NaCl were found to exhibit lower values of the parameters examined than those recorded in the first experimental week (Figure 2). Moreover, they showed mild depressions and brown spots on the leaf, whereas brownish-black edges were detected on the leaves of some plants.





Figure 2. Effect of increasing NaCl concentrations on the characteristics of pansies

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At the end of the experiment, a decrease in the parameter values measured was recorded. The number of flowers decreased (a large number of plants did not have flowers at the end of the experiment, but new buds appeared), whereas the length and width of leaves remained within the optimal range. In the eighth experimental week, the plants treated with 3, 5 and 7 g/l NaCl exhibited changes in both leaves and flowers, i.e. wilting and drying of flowers with the emergence of new buds. Moreover, the vitality and decorativeness of all the plants were greatly reduced, which is important from the horticultural and ornamental perspectives (Figure 3).



Figure 3. Pansy seedlings at the end of the experiment

After the experiment, measurements of the electrical resistance (Table 2), pH value (Table 3) and electrical conductivity (Table 4) of soil extracts were performed.

Control	Treatment 1	Treatment 2	Treatment 3
	3 g/l NaCl	5 g/l NaCl	7 g/l NaCl
0.12	0.32	0.38	0.44

On the basis of the results shown in Table 2, it can be concluded that the highest percentage of salt was determined in the soil extract treated with 7 g/l NaCl (T3), whereas the lowest percentage of salt was determined in the control soil extract (0.12%).

Table 3. pH value of the samples tested

Control	Treatment 1	Treatment 2	Treatment 3
	3 g/l NaCl	5 g/l NaCl	7 g/l NaCl
7.01	7.30	7.31	7.25

Table 3 shows the average pH values of the tested substrate samples. The pH values were measured in a saturated soil trap and ranged from 7.01 to 7.31, indicating a neutral to weak alkaline reaction of the substrate. The T2 plants (treated with 5 g/l NaCl) were found to exhibit a maximum pH value of 7.31. According to Nawaz et al. (2010), high salt levels not only exert damaging effects on plants, but also increase the pH level of the soil. Most crop plants do not grow well at high pH levels.

Control	Treatment 1	Treatment 2	Treatment 3
	3 g/l NaCl	5 g/l NaCl	7 g/l NaCl
0.53	2.8	3.67	6.54

Table 4. ECe values of the substrate water extract at 25 °C (mS/cm)

The electrical conductivity values of the saturated substrate extracts are shown in Table 4. On balance, the highest value of ECe was recorded in the treatment with 7 g/l NaCl (6.54 mS/cm). At this ECe value, plants will suffer a significant reduction in growth, thus requiring adequate care to ensure the desired level of their vitality and decorativeness.

DISSCUSION

The stress induced by saline soils affects the plant metabolism and the outcome of agricultural production in a lot of different ways (Marschner, 1995). The decreased intensity of the plant growth, as a consequence of the presence of a higher concentration of salt, can first be noticed in mass and leaf area of the treated plants (Lauchli and Epstein, 1990). A number of authors have dealt with the use of ornamental plants in landscapes (Cassaniti et al., 2009; Marosz, 2004). Salinity and alkalinity are of paramount importance to the quality of water and, if not carefully monitored, they can reduce the growth and development of plants up to 50% (Roberts, 1991). The results of our study show that concentrated water adversely affects the growth and development of pansies. In their study on the influence of salinity on pansies and impatients in different growth media, Kuehni and Morales (1998) also reported necrotic areas on the leaf, as well as decreased numbers of leaves and flowers. The research of Savved et al. (2014) argues similar changes in the Mexican marigold (*Tagetes erecta* L.) to those recorded in pansies under the influence of salt, especially the root system damage. The salt effect on the water regime of the plants is considered to one of the main causes of decreased plant growth. The osmotic pressure of the soil solution increases with the increase in NaCl concentration and consequently. water uptake by plants is often prevented. As a results, water becomes unavailable for plants despite its presence. This phenomenon is called 'physiological drought' (Avers and Westcot, 1976). High concentration of salts aggravates water absorption and leads to decrease of absorptive root area, which is necessary for water uptake. Along with this, the leaf area increases, which reduces the whole

transpiration process. The immediate plant response to such effects is stomatal closure, which was found in many other plant species (Hasegawa et al., 2000). In saline conditions plants shorten their vegetation, their water regime is disturbed and the yield is reduced as it was shown for pea by Maksimović et al. (2008 and 2010). When it comes to soil salinity, safflower (*Carthamus tinctorius* L.) is considered to be a tolerant species, although relatively low concentrations of NaCl affects its growth, water regime and metabolism (Daničić et al., 2016). Some plant species are more tolerant to high concentrations of salt than others. The type and age of the plant, as well as the amount of salt present in the soil and the duration of the plant's exposure to salt concentrations, influence the uptake of salt by the plant, as well as its growth and development. These parameters also affect the ability of plants to absorb nutrients and minerals, which are vital to their normal growth and development

CONCLUSION

The results obtained in the present study indicate that large amounts of salt in the autumn and winter months damage the pansy. Increased salt concentrations exert a negative impact on the aesthetic value of pansy, thus reducing its vitality and decorativeness. Although the pansy is considered a very durable plant, it is still susceptible to high salt concentrations, which adversely affect its growth and development. The parameter values obtained were found to be lower in the pansy plants treated with 5 and 7 g/l NaCl. These plants mostly exhibited changes on the leaf surface such as depressions, brown spots, yellowing and curling, whereas their flowers wilted and fell off. The results of the pedological analysis performed argue that the highest pH value of the soil solution was determined in the treatment with 5 g/l NaCl (T2). The pansy seedlings treated with 7 g/l NaCl were found to exhibit the highest percentage of water-soluble salts and the highest ECe value of the water extract.

To ensure healthy and decorative pansy seedlings during ice and snow melting, low concentrations of salt should be used in the flower bed area, as well as different materials (namely plastic or fabric) for covering the seedlings to conserve their vitality and decorativeness. Therefore, pansies can be recommended for use in salt-affected flower beds.

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УТИЦАЈ ЗАСЛАЊИВАЊА НА РАСТ И РАЗВОЈ ЉУБИЧИЦЕ (*Viola x wittrockiana* Gams.)

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РЕЗИМЕ: Сврха овог рада је да се утврди утицај заслањивања земљишта на раст и развој љубичице (Viola x wittrockiana). Циљ истраживања је сагледавање начина употребе љубичице у јесење-зимском аспекту, као и правилнији одабир површине на којој ће се гајити. За потребе експеримента коришћено је укупно 40 садница љубичице подељених у четири групе од по десет садница. Третмани су обележени под називом контрола (K), третман са 3 g/l NaCl (T1), третман са 5 g/lNaCl (T2), третман са 7 g/l NaCl (T3). Концентрована вода је коришћена за заливање третмана Т1, Т2 и Т3, чије су промене праћене сваке недеље. Током осмонедељног експеримента измерене су следеће квантитативне и квалитативне особине садница љубичице: висина, број листова, број цветова, пречник цвета, дужина листа, ширина листа као и виталност и декоративност. Резултати добијени током истраживања показују да љубичице могу да поднесу веће количине соли од 5 и 7 g/l NaCl, али са негативним последицама на њихов раст, развој, виталност и декоративност.

КЉУЧНЕ РЕЧИ: декоративност, љубичице, заслањивање, натријум-хлорид (NaCl), третмани, виталност

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EFFECT OF LOW NaCl CONCENTRATIONS ON THE WATER RELATIONS OF RAPESEED

ABSTRACT: The experiment was carried out in order to examine whether relatively low concentrations of NaCl (0.2, 0.6 and 1.2 g NaCl/L in the nutrient solution), which may appear in agricultural soils or irrigation waters, affect physiological processes in young rapeseed plants (cultivar Slavica) grown under semi-controlled conditions, on a nutrient solution. The experiment was done in 7 replications, with 8 plants per replication. Increased salinity had no influence on adaxial density of stomata, while abaxial density of stomata and specific leaf area declined with an increase in NaCl concentration in the nutrient solution. The intensity of transpiration also declined with an increase in NaCl concentration in the nutrient solution. Even though plant growth was not significantly affected by applied concentrations of NaCl, it is evident that the impact of salts is complex and it is necessary to study whether there are significant changes in plant metabolism.

KEYWORDS: Brassica napus L., salt stress, density of stomata, intensity of transpiration, specific leaf area

INTRODUCTION

The salinity of the soil solution is one of the most important abiotic external environmental conditions affecting the quality and yield of cultivated plants in semiarid and arid regions. In these conditions, evapotranspiration often exceeds the amount of precipitation, which leads to an increase in salt concentration. Likewise, a great contribution to this problem is a low amount of rainfall, parent substrate and a high concentration of salts in the irrigation water within agricultural areas. Salinization process control, through ameliorative measures for soil repairing, and irrigation improving are not cost-effective and are often short-term solutions (Arzani, 2008). Salinization can be done due to

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either natural processes (primary salinization) or anthropogenic action (secondary salinization) (Ghassemi et al., 1995).

Even if there is a major concern in increasing our food production with the world population's alarming growth rate (expected to reach 9.1 billion people by 2050), at the same time there is a significant decrease in the availability of arable land as a result of urbanization and soil degradation. Also, salinity represents one of the main obstacles that limit the expansion of agricultural areas or the increase in agricultural production for many crops (Setter and Waters, 2003).

Saline environments affect plant growth in different ways, such as a reduction of water uptake, an accumulation of ions to toxic levels and a reduction of nutrient accessibility. In some extensive reviews concerning strategies for overcoming the salinity problem, two primary lines of action were emphasized: reclamation of salt-affected soils by chemical amendments, and alternatively, the saline soils used to grow salt-tolerant plants (Ashraf and McNeilly, 2004).

According to FAO (1997), more than six percent of soils in the world contain excessively high concentrations of salts or sodium. The increased concentration of sodium is present in more than 400 million hectares, which is about 3.4% of arable soils. Salinity may reduce the crop yield by disturbing water and the nutritional balance of plants (Islam, 2001). Even if concentrations of salts in soils are not increased to the extent that it inhibits the growth of plants, it may cause a reduction in yield and changes in the quality of plant tissue, also affecting the market value of agricultural products.

Water availability and nutrient uptake by plant roots are limited because of high osmotic potential and toxicity of sodium (Na⁺) and chlorine (Cl⁻) ions (Kumar, 1995). Severe changes in ions and water homeostasis lead to molecular damage, growth disorder and even death (Zhu, 2001).

Furthermore, in agricultural practice, obtaining sufficient amounts of irrigation water of good quality is not always possible. Often intensively mineralized waters are used as well as processed municipal wastewaters which contribute to the accumulation of salts (Kalavrouziotis et al., 2010). The final outcome of the influence of impurities in the water for irrigation on the metabolism of vegetables depends on the nature of the substance, the time of exposure and the ability of plants to adapt. This ability is genetically determined but it also depends on the combination of agro-ecological conditions in which the plant develops (Maksimović and Ilin, 2012).

In Serbia, rapeseed is the most important source of oils, besides sunflower and soybean. The most common adverse effect of salinity on the crop of *Brassica* is the reduction in plant height, size, and yield as well as deterioration of the product quality (Zamani et al., 2010). Tolerance of rapeseed to salt stress is a complex trait greatly modified by cultural, climatic and biological factors (Mahmoodzadeh, 2008).

There are significant differences in salt tolerance between plant species and genotypes and this goes for the ability to tolerate water deficiency (Lukovic et al., 2009). During stress conditions, plants need to maintain internal water potential below that of soil and maintain turgor and water uptake for growth. This requires an increase in osmotica, either by uptake of soil solutes or by the synthesis of metabolic (compatible) solutes.

The adverse effect of salt stress is expressed on the whole plant level and appears during all developmental stages. However, tolerance with respect to salt stress varies at different plant developmental stages and also from species to species. The plant responds to salt stress by various processes that function in coordination to balance cellular hyperosmolarity and ion disequilibrium. The plant's ability to tolerate salt is dependent on multiple biochemical pathways that lead to production of osmotically dynamic metabolites, free radicals, and specific proteins to manage ion and water flux, thus providing support to scavenging oxygen radicals and in turn maintaining ion homeostasis. Salt stress has shown to affect many intracellular substances, like nucleic acids, proteins, carbohydrates and amino acids (Ahmad et al., 2010).

MATERIALS AND METHODS

The objective of this study was to assess the effect of different concentrations of NaCl (0 [control]; 0.2; 0.6 and 1.2 g/L) on rapeseed (*Brassica napus* L.), cultivar Slavica, grown in the semi-controlled conditions of a greenhouse. Analyses of plant growth and water regime (transpiration and percentage of dry matter), were done 4 weeks after exposure to NaCl.

Seeds were sown in the Petri dishes filled with sand. Previously the sand had been sieved, then sterilized in the furnace at 600 °C. Germination took place in the incubator, in the dark, at 25-26 °C.

During germination, irrigation has been performed with deionized water. After germination, plantlets were transferred into the pots with nutrient solution. Plantlets were transplanted into the pots with a volume of 700 ml filled with $\frac{1}{2}$ strength Hoagland nutrient solution (Hoagland and Arnon, 1950). The nutrient solution was regularly aerated and weekly replaced with a fresh one in order to prevent contamination by root excretions, changes of pH (pH was between 5.5 and 6) and to provide plants with all the nutrients.

After 14 days, to the nutrient solution, the following amounts of NaCl were added to the nutrient solution: 0 (control): 0.2; 0.6; or 1.2 g NaCl/L. The experiment was set up in 7 replications, with 8 plants per replication. Four weeks after the beginning of treatment the following analyses were done: measurement of biomass, total leaf area, specific leaf area (dry mass/leaf area), the intensity of transpiration and adaxial and abaxial prints of leaf epidermis were taken in order to analyze stomatal features.

Transpiration intensity (It) was measured gravimetrically during 3 consecutive days, at 8, 10, 12 and 14h. Leaf area was measured with the automatic photoelectric Ci-203 laser area meter (CID Bio-Science Inc).

Statistical analysis of data was performed by Statistica 13.3 software. The analysis of variance (ANOVA) was performed at 95% confidence level.

RESULTS AND DISCUSSION

The production of the dry mass of leaves, stems and roots per plant and total leaf area were not significantly different among plants treated with 0.2, 0.6 and 1.2 g NaCl/L and control plants (P<0.05) (Figure 1). Growth analysis is a fundamental characteristic to study plant response to environmental stress (Salem et al., 2013). Decreased growth rate due to the increased concentrations of salts, in general, is manifested as decreased biomass, leaf area and yield (Läuchli and Epstein, 1990; Allen, 1994). Munns (2002) explains that during initial exposure to salinity, plants experience water stress, which in turn reduces leaf expansion. Our results were in contrast to these claims, which bring us to the conclusion that applied concentrations were not high enough to cause significant reduction of growth of young rapeseed plants.



Figure 1. Impact of NaCl on the dry mass of leaves, stems and roots.

Leaf area and stomatal density play an important role in maintaining water balance, and gas exchange is regulated by their aperture and density, two traits that vary in response to environmental conditions, such as water stress and salinity. In young rapeseed plants, the density of stomata was not significantly affected by increased salt concentration, except for the density of stomata on the abaxial side of leaves in the presence of 0.2 g NaCl/L (Figure 2). The ratio of number of stomata per number of epidermal cells is genetically determined and therefore changes in the density of stomata suggest that the size of epidermal cells is altered. The final result depends on both plant species, concentration of salts and overal agroecological conditions. In tomato, the

presence of salt reduced stomatal density (Romero-Aranda et al., 2001), but increased it in pea (Maksimović et al., 2010).



Vertical bars denote 0.95 confidence intervals

Figure 2. Impact of the NaCl on a density of stomata. Different letters indicate a significant difference at 5% probability level

The specific leaf area (dry mass/leaf area) declined with an increase of NaCl concentration in nutrient solution (Figure 3). Plants treated with 0.6 and 1.2 g NaCl/L had significantly lower specific leaf area (mg/cm²) than the control, even though differences in dry mass and total leaf area did not differ significantly among treatments. Salinity may cause anatomical changes in the leaves of many plant species. For example, the epidermis and mesophyll leaves of beans, cotton, and *Attriplex* become thick, the length of palisade mesophyll cells and diameter of spongy mesophyll cells increase and the thickness of palisade and spongy layers increase as well (Longstreth and Nobel, 1979). In some other plant species, adverse effects were recorded. In spinach leaves, the presence of salt reduces the intercellular spaces (Delfine et al., 1998).



Figure 3. Impact of NaCl on the specific leaf area (mg/cm²). Different letters indicate a significant difference at 5% probability level.

The intensity of transpiration in the plants treated with two higher concentrations of NaCl was significantly lower (Figure 4). With increased salinity, water adsorption by the root is more difficult, which leads to increased transpiration (Ayers and Westeat, 1976). In this experiment, the results were the opposite. The intensity of transpiration was higher when plants were treated with 0.2 g NaCl/L but not significantly compared to the control, while plants treated with two higher concentrations had significantly lower intensity of transpiration compared to the control and plants treated with 0.2 g NaCl/L. It can be explained as dilution – one of the mechanisms by which plants protect their cells from harmful effects of high concentrations of salts; then, increasing water retention in the tissues of the plant further reduces transpiration. In this experiment, reduction in the intensity of transpiration is in line with the reduction in specific leaf area. The results were in correspondence with the results obtained by Ashraf and Neilly (2004) who found that leaf water potential and evapotranspiration significantly decreased with increasing salt concentration in six species of the Brassica genus. Consequently, some phenophases may be shortened, and the uptake and distribution of essential elements in both semicontrolled and field conditions altered (Maksimovic et al., 2010).


Figure 4. Impact of NaCl on the intensity of transpiration ($g H_2O/dm^2/h$).

CONCLUSION

In the present study, low concentrations of NaCl (0 [control], 0.2, 0.6 and 1.2 g/L) did not significantly affect the vegetative growth of young rapeseed plants. Production of the dry mass of leaves, stems and roots was not significantly changed probably because the plants were exposed to the treatment not longer than four weeks.

The lowest applied concentration of NaCl (0.2 g/l) did not significantly change specific leaf area and intensity of transpiration, but reduced stomatal density of the abaxial side of leaf blade. On the contrary, higher concentrations of NaCl (0.6 and 1.2 g/L) significantly decreased specific leaf area and intensity of transpiration. Therefore, the observed changes suggest that even relatively low concentrations of NaCl lead to significant changes in rapeseed water relations, even before the changes in biomass production become evident.

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ЕФЕКАТ НИСКИХ КОНЦЕНТРАЦИЈА NaCl НА ВОДНИ РЕЖИМ УЉАНЕ РЕПИЦЕ

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РЕЗИМЕ: Експеримент је спроведен да би се испитало како релативно ниске концентрације NaCl (0,2, 0,6 и 1,2 g NaCl/L у хранљивом раствору), које се могу појавити у пољопривредним земљиштима или водама за наводњавање, утичу на физиолошке процесе у младим биљкама уљане репице (сорта "Славица") у полуконтролисаним условима. Експеримент је изведен у седам понављања, са по осам биљака у понављању. Повећана количина соли у раствору није имала утицаја на густину стома на горњој (адаксијалној) страни листа, док се на абаксијалној страни густина стома као и специфична површина листа смањивала с порастом концентрације NaCl у хранљивом раствору. Интензитет транспирације се, такође, смањио с порастом концентрације NaCl у хранљивом раствору. Иако примењене концентрације NaCl нису значајно утицале на раст биљака, очигледно је да је утицај соли комплексан чак и при примени нижих концентрација и да је неопходно проучити постоје ли значајније промене у метаболизму уљане репице.

КЉУЧНЕ РЕЧИ: *Brassica napus* L., сони стрес (NaCl), густина стома, интензитет транспирације, специфична лисна површина

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THE ACCUMULATION OF K⁺, Ca²⁺, Na⁺ AND P IN Sinapis nigra L. GROWN IN THE PRESENCE OF NaCl AND SILICON

ABSTRACT: Saline stress is recognized as one of the most important abiotic stresses for plants in some regions of the world. Recent studies showed that silicon (Si) can play an important role in plant resistance to environmental conditions, such as excessive salinity. In the present experiment, *Sinapis nigra* L. was exposed to two levels of salinity (50 and 100 mM NaCl). To study the effect of Si addition on the concentrations of K^+ , Ca^{2+} , Na^+ , and P, in the vegetative parts of plants, 5 mM of Si in the form of fertilizer (Siliplant) was added to the nutrient solutions. The experiment was conducted in semi-controlled conditions. The results showed that both salinity levels significantly increased the concentration of Na⁺ in leaves, stems, and roots, whereas the concentration of Ca^{2+} and K⁺ were decreased (up to 55% and 90% in the presence of 100 mM NaCl). The addition of Siliplant to the saline nutrient solution (50 mM NaCl) was efficient in decreasing Na⁺ accumulation in leaves and stem, while in roots more alleviating effect of Si addition was pronounced in the presence of 100 mM NaCl (Na⁺ concentration decreased by 50%). The presence of Si in 50 mM NaCl containing nutrient solution enhanced the accumulation of K^+ in leaves and stems, while Ca^{2+} concentration was decreased, or the addition of Si to a nutrient solution had a negligible effect. Based on the results of the present study, the application of Si in the form of fertilizer was found more efficient in the presence of lower salt concentration (50 mM NaCl). Therefore, higher doses of the fertilizer should be considered and tested in case of higher NaCl concentrations in the substrate.

KEYWORDS: black mustard, NaCl, nutrient concentration, Si

INTRODUCTION

Sinapis nigra L. or the black mustard is a plant cultivated mostly for food and medicinal purposes. It belongs to the Brassicaceae family and is native and grown in arid and semi-arid areas of the world where the drought and salinization can adversely affect the growth and quality of cultivated plants

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which can eventually decrease their yield and economic value (Abbaoui et al., 2018). In recent decades, the problem of salinization is aggravated by climate change and the use of highly mineralized irrigation water as well as improper soil management (Taiz and Zeiger, 2014).

The excess of salts, such as NaCl and Na₂SO₄ is a global problem that affects approximately 20% of arable land and reduces crop production significantly (Qadir et al., 2014). Na⁺ is beneficial for plants, but in saline conditions, it is accompanied by Cl⁻. Although Cl⁻ can positively affect plant metabolism, it is proven worldwide that Cl⁻ mostly exceeds its level to toxic levels. Excessive salt amounts in the soil solution can cause many metabolic disorders in plants, which eventually lead to a reduction of growth and loss of yield (Munns et al., 2008). Growth reduction is attributed mostly to ion toxicity and low water potential (Munns and Termaat, 1986). In addition, salinity can affect nutrient balance in plant tissues. A number of studies showed an increase of Na⁺ and a decline in K⁺, Ca²⁺ and Mg²⁺ concentration in plants. However, the extent of nutrient disorders in plants, as a consequence of the increased concentration of NaCl in soil solution, depends on plant species and levels of salinity (Khan, 2001; John et al., 2003).

Silicon (Si) is the second most abundant mineral in the earth's crust. The silicon content of soils can vary from <1 to 45% dry weight (Sommer et al., 2006). Silicon can leach out, redistribute, or accumulate in soils during soil development. Although silicon is abundant in soil, most of it cannot be absorbed directly by plants. Plant root generally takes up silicon in the form of soluble silicic acid [Si(OH)4] (Mitani and Ma, 2005), an undissociated molecule that is normally present at 0.1–0.6 mM in the soil solution at most naturally occurring pHs (pH 1–9) (Ma and Yamaji, 2006). Although silicon limitation does not affect plant growth, its application increases the yield of many crops (Mcginnity, 2015). Many roles of Si, including the establishment of nutrient balance in plants. reduction of toxicity caused by an excess of some elements in plant tissues (such as Na⁺) and improvement of mechanical resistance of plant tissues, under saline conditions, were recognized so far (Bybordi and Tabatabaei, 2009). Nevertheless, the effect of Si addition on the nutrient status of medicinal and food plant such as *Sinapis nigra* L. in saline conditions is scarce (Ali et al., 2013).

In recent years, the Brassicaceae family became a focus on the growing interest as studies have shown that high consumption of green parts (shoot) and seeds of these crops reduces the risk of a great number of health problems (Abbaoui et al., 2018). The high consumption level of Sinapis herb reduces the risk of a great number of cancers, particularly cancers of the gastrointestinal tract, lung, bladder and prostate (Johnson, 2018). The ability of Sinapis species to reduce health risks depends mostly on the biochemical quality of a plant (Boscaro et al., 2018). Therefore, the present study aims to examine the effect of Si application on concentrations and distribution of K⁺, Ca²⁺, Na⁺ and P in leaves, stems, and roots of *Sinapis nigra* L. in saline conditions.

MATERIALS AND METHODS

The experiment was conducted in a glasshouse in semi-controlled conditions. *Sinapis nigra* L. seeds were germinated in previously sterilized sand. The seeds were subjected to the treatments with NaCl and Si, as presented in Table 1, from the beginning of the experiment. Si (7%) was applied in the form of fertilizer Siliplant Univerzalni (EkoPlant d.o.o. Beograd) in amounts recommended by the producer (5 mM). After germination, healthy and uniformed seedlings were transferred to black pots (V= 750 mL), filled with daily aerated ½ strength Hoagland solution (1/2 H) (Hoagland and Arnon, 1950), which contained salt and Si (Table 1). The nutrient solution was replaced weekly. Thirty days after the planting, the concentrations of Na⁺, K⁺, Ca²⁺ and P were assessed.

The harvested plants were divided into leaves, stems, and roots measured and dried at 70 °C to constant weight. Sodium, potassium, and calcium concentrations were assessed by flame photometry. Phosphorus concentration was assessed by ammonium vanadate molybdate method (MAFF/ADAS, 1986).

Statistical analyses of the data were carried out using Statistica 13 statistical software. All data were subjected to the analysis of variance and comparisons of means were performed using Fisher's least significance test (LSD) at p < 0.05.

Principal component analysis (PCA) was conducted by XLStat software version 2014.5.03 (Addinsoft). PCA was based on Pearson's correlation coefficient.

Treatment	Label	NaCl concentration (mM)	Siliplant concentration (mM)
1.	1/2 H (Control)	0	0
2.	½ H+Si	0	5
3.	1/2 H+50 NaCl	50	0
4.	1/2 H+50 NaCl+Si	50	5
5.	1/2 H+100 NaCl	100	0
6.	¹ / ₂ H+100 NaCl+Si	100	5

Table 1. The composition and labels of nutrient solutions used in the experiment

RESULTS AND DISCUSSION

The results presented in Figure 1 show that both salinity levels (50 and 100 mM NaCl) significantly increased Na⁺ concentration in leaves, stems, and roots of *Sinapis nigra*, relative to control and 1/2 H+Si (in leaves and roots up to 76% and 80%, respectively). The addition of silicon to 50 mM salt-containing nutrient solution decreased the Na⁺ concentration in leaves and roots by

13% and 11%, respectively. The presence of Siliplant in a solution that contained 100 mM NaCl significantly decreased the accumulation of Na⁺ in roots of black mustard, with respect to the same treatment without Si (approximately 50%). The addition of Siliplant to solutions that contained both salt concentrations (50 and 100 mM NaCl) significantly increased the concentration of K⁺ in leaves (by 39% and 40%, respectively), while in stems, its concentration was significantly increased by the addition of Si to 50 mM NaCl (57%). However, in roots, the addition of Si decreased the K⁺ accumulation by 60% in the presence of 50 mM NaCl, and by 66% in the presence of 100 mM NaCl, relative to the same salt treatments without Si (Figure 1).

Excessive concentrations of NaCl in the growing substrate can be a major limiting factor for plant growth and productivity. Besides the higher osmotic potential of soil solution, which can result in water deficit within the plant tissues, higher concentrations of Na⁺ and Cl⁻ can cause ion toxicity and secondary stress such as nutritional deficiency (Yue et al., 2012). In general, increased salt concentration usually results in an increase in Na⁺ accumulation and a decrease in accumulation of the other cations, like K⁺ and Ca²⁺ (Wang and Han, 2007). Higher levels of Na⁺ are injurious to cellular metabolism and lead to reduced plant growth and imbalanced nutrient status. Under salt stress, plants expend additional cellular resources to maintain a high concentration of cytosolic K⁺ and low concentration of Na⁺ (Mahajan and Tuteja, 2005).

The significantly lower concentration of Ca^{2+} in all three tissues of the black mustard in the presence of NaCl with respect to the control (1/2 H) (up to 55% in leaves in the presence of 50 mM NaCl) was obtained in the present experiment (Figure 1). The addition of Si to a saline nutrient solution had either negligible or adverse effects on Ca^{2+} accumulation in tissues of treated plants. In leaves, the addition of 5 mM of Si to 100 mM NaCl significantly decreased Ca^{2+} concentration (17%), relative to the treatment without Si. In the roots of black mustard both Ca^{2+} and P accumulated similarly, but to different extents. The addition of silicon to both saline nutrient solutions (50 and 100 mM NaCl) significantly decreased the concentration of Ca²⁺, with respect to Si-deficient treatment (by 53% and 59%, respectively). The same effect of Si addition to 50 and 100 mM NaCl solution was observed in the case of P (by 60% and 70%, respectively). Different studies on the plant response have shown that salinity causes modifications of the tissue levels of elements like Ca²⁺ and P. Hellal et al. (2012) reported increased P, and Ca²⁺ concentrations in the shoots and seeds of fava bean cultivated under salt stress when Si was supplied. In addition, silicon enhanced the P and Ca²⁺ accumulation in leaves and roots of aloe plants (Li et al., 2015). Application of Si significantly increased the Ca^{2+} concentration in shoots of cucumber plants exposed to salinity. However, it had no effect on the shoot Ca²⁺ concentration of plants grown under non-saline conditions (Khoshgoftarmanesh et al., 2014).



Figure 1. The concentration of K⁺, Na⁺, P and Ca²⁺ in dry weight of leaves, stems, and roots of *Sinapis nigra* L. exposed to NaCl and Si. Vertical bars denote 0.95 confidence level.

The presence of NaCl in the nutrient solution altered the distribution of K^+ , Na⁺, and Ca²⁺, while the Si addition was most efficient in the case of lower salt concentration (50 mM NaCl) (Figure 2). Although both K^+ and Ca²⁺ distribution in leaves, stems, and roots of black mustard were severely disrupted in the presence of 50 and 100 mM NaCl in the solution, only the addition of Si to less saline nutrient solution (50 mM NaCl), was efficient in alleviating saline stress. The distribution of K^+ in leaves was increased by 50% in the presence of Si in 50 mM NaCl-contained solution, with respect to the same treatment without Si. Significantly decreased distribution of Na⁺ in roots of black mustard in the presence of $\frac{1}{2}$ H+100 NaCl+Si, relative to $\frac{1}{2}$ H+100 NaCl (50%) was recorded.

Higher NaCl concentrations in the substrate may alter the balance of ions in plants. The competition between Na⁺ and K⁺ uptake affects the K⁺/Na⁺ ratio. In the present experiment, under control (1/2 H), the cell maintained a high K⁺/Na⁺ ratio (Figure 3). However, in the presence of NaCl in the growing medium (both with and without Si), due to the similarity of K⁺ and Na⁺ radii, it was difficult for plants to distinguish between these two ions. The higher influx of Na⁺ through K⁺ pathways might be the main reason for the decreased K⁺/Na⁺ ratio in the presence of both Si-treated and pure salt treatments (Blumwald, 2000).



Figure 2. The distribution of K⁺, Na⁺, Ca²⁺ and P in leaves, stems and roots of *Sinapis nigra* L. exposed to NaCl and Si.



Figure 3. The K⁺/Na⁺ ratio in leaves, stems and roots of *Sinapis nigra L*. exposed to NaCl and Si. Vertical bars denote 0.95 confidence level.

In the present experiment, the data were also analyzed using Principal Component Analysis (PCA). PCA was performed by 12 variables (percentages of Na⁺, K⁺, Ca²⁺ and P in leaves, stems, and roots of black mustard). As a result of PCA, two principal components (PC1 and PC2) captured most of the variation (69.47% and 21.52%, respectively) (Figure 4). Therefore, the cumulative of PC1 and PC2 was 90.99%. In addition, PCA resulted in a distinct separation between Control and $\frac{1}{2}$ H+Si on one side of the biplot (left) and salt ($\frac{1}{2}$ H+S0 NaCl and $\frac{1}{2}$ H+100 NaCl) and silicone ($\frac{1}{2}$ H+50 NaCl+Si and $\frac{1}{2}$ H+100 NaCl+Si) treatments on the other side of the biplot (right). The close association of Control and $\frac{1}{2}$ H+Si reflects their similar effect on the measured variables, which was confirmed by the LSD test (Figure 1).

Based on the angles between the vectors of the biplot, two main groups of variables were distinguished. The groups consist of positively associated variables (they form the angles less than 90 degrees). The left group consists of a percentage of K⁺ and Ca²⁺ in leaves, stems, and roots, as well as the percentage of P in roots. The second group (on the right side of the biplot) is comprised of a percentage of Na⁺ in all three tissues of black mustard (leaves, stems and roots) and percentage of phosphorus in stems and leaves. The percentage of Na⁺ is strongly associated with both of the salt treatments ($\frac{1}{2}$ H+50 NaCl and $\frac{1}{2}$ H+100 NaCl), which is supported in Figure 1 (Na⁺ concentration in leaves, stems and roots significantly increased in saline nutrient solutions, relative to control). On the contrary, according to the LSD test, percentages of K⁺ and Ca²⁺ in all three tissues were adversely affected by the salt treatments (their concentrations were significantly decreased with respect to control and $\frac{1}{2}$ H+Si), which PCA reflects.



Figure 4. Principal component analysis (PCA) of mineral elements. Biplot is presented with first and second principal components (PC1 and PC2). L – *leaves*; S – *stems*; R – *roots*.

CONCLUSION

The present study assessed the effect of Si fertilization on Na⁺, K⁺, Ca²⁺, and P concentration and distribution in tissues of *Sinapis nigra* L. in saline conditions (50 and 100 mM NaCl). The results revealed that Si application caused an increase in K⁺ concentration in leaves and stems relative to Si absent treatments. However, the Na⁺ concentration was significantly decreased only in roots after the addition of Si to the higher salt-contained solution (100 mM NaCl). Si presence in saline nutrient solutions resulted in adverse effects or no effect at all on Ca²⁺ concentration, as well as on the concentration of P. Based on our findings, the application of higher doses of Si fertilizer Siliplant should be considered and tested further.

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АКУМУЛАЦИЈА К⁺, Са²⁺, Na⁺ И Р КОД ЦРНЕ СЛАЧИЦЕ (*Sinapis nigra* L.) У ПРИСУСТВУ NaCl И СИЛИЦИЈУМА

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РЕЗИМЕ: Заслањеност је један од најважнијих абиотичких чинилаца који може изазвати стрес и негативно утицати на развој биљака. Силицијум (Si) је елемент који има значајну улогу у повећању отпорности биљака на неповољне услове, међу којима је и заслањеност супстрата. У раду је испитан утицај додавања силицијума заслањеном хранљивом раствору на концентрацију и дистрибуцију Na⁺. К⁺, Са²⁺ и Р у листу стаблу и корену црне слачице (Sinapis nigra L.). Додато је 5 mM силицијума у облику ђубрива (Siliplant) у хранљиви раствор који је садржао два нивоа заслањености (50 mM и 100 mM NaCl). Експеримент је спроведен у полуконтролисаним условима у стакленику. Резултати су показали да су обе концентрације соли у раствору значајно повећале концентрацију Na⁺ у ткивима црне слачице, док су концентрације K^{+} и Ca²⁺ биле значајно смањене. Додатак силицијума хранљивом раствору који је садржао 50 mM NaCl генерално је смањило концентрацију Na⁺ у надземном делу слачице, док је значајно смањење концентрације Na⁺ добијено у корену биљака, додатком Si раствору који је садржао 100 mM NaCl (50%). Присуство Si у раствору који је садржао нижу концентрацију соли (50 mM) повећало је концентрацију K^+ у надземном делу слачице, док је концентрација Ca^{2+} била смањена, или додатак Si није имао никакво дејство. На основу резултата експеримента, примена Si у облику ђубрива била је ефикаснија у случају нижих концентрација соли (50 mM). Због тога је потребно испитати и тестирати примену већих доза (већих од 5 mM) Siliplanta, у случају виших концентрација соли у супстрату (100 mM NaCl).

КЉУЧНЕ РЕЧИ: Sinapis nigra, NaCl, концентрација елемената, силицијум (Si)

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6.2. Write the names of living organisms using Italics font style.

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6.4. Chemical formulas and complex equations should be drawn and pre¬pared for photographic reproduction.

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8.3. Above each table, write a capture with table explanation.

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