Use of vermitea for removing pesticides from soil

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HIGHLIGHTS

- Vermitea, a liquid extract from vermicompost, contents high biological activity and dissolved organic carbon.
- Vermitea may be used as a bioremediation method to enhance pesticides degradation in soil.
- High concentration of vermitea added to soil may have a negative effect on soil biological activity.

SUMMARY

Tea obtained from vermicompost (vermitea) is currently used as liquid fertilizer but its potential to degrade pesticides in soil has never been studied. In the current study we evaluate the effect of tea from vermicompost of olive cake to degradate pesticides of different hydrophobicity: imidacloprid, diuron, tebuconazole and oxyfluorfen. For the degradation study, a non-amended soil and soils amended with two dosage of vermitea were incubated for two months. Results indicate that despite the dehydrogenase activity was higher in soil amended than in non-amended soil, addition of vermitea to soil does not significantly increase the soil ability to degrade the pesticides studied. In soil amended with vermitea at high dosage, the remaining concentration of diuron and oxyfluorfen at the end of the incubation time was significantly ($P < 0.01$) higher than in non-amended soil suggesting a negative effect of the vermitea on the microbial activity. These preliminary results indicate the necessity of improving the vermitea elaboration process adding nutrients during the aeration to increase its biochemical quality and the content of microorganisms to favor their potential to degrade organic contaminant such as pesticides.

INTRODUCTION (AND OBJECTIVES)

The presence of pesticides in the environment and their impact on human health and ecosystems are big concerns all over the world. In the majority of the world´s countries, standards to preserve the quality of the hydric resources have been implanted. The European Union legislation has initiated more stringent data requirements concerning ground and surface water contamination by pesticides (Directivas 2000/60/EC, 2006/118/EC). In order to reach the standards of quality of waters, and to fulfill those normative, it is necessary to develop techniques to reduce and prevent the origin of the contamination. Current research is focused on developing environmental friendly and low cost bioremediation strategies. Tea

obtained from vermicompost, also known as vermitea, which is used to enhance agricultural productivity, may be applied to soil as a detox agent to minimize pesticide contamination. Vermitea is a liquid extract obtained from the mature vermicompost by aeration. It contains soluble nutrients and a diversity of beneficial microorganisms that increase the soil quality and the plant fertility [1, 2]. Nowadays, there is an increasing interest for using vermitea to increase the agriculture productivity and reduce the use of chemical fertilizer. However, the application of vermitea to minimize contamination causes by organic contaminants has been scarcely studied. Chiang (2013) introduced the use of vermitea as a remediation tool in the context of oil contaminated soil. The soil application of vermitea causes an increase of the microbial activity and thus it may enhance the microbial degradation of organic compounds such as pesticides. Also, the high content of vermitea´s dissolved organic carbon (DOC) may cause desorption of the sorbed fraction of organic contaminants to the soil solution [4] increasing their bioavailability and enhancing their degradation. Thus, vermitea may become an important tool for bioremediation strategies and management risk, especially for most hydrophobic organic contaminants which should render more adsorbed into the solid phase and thus less bioavailable but with high affinity for DOC [5, 6].

The aim of the current study is to test tea obtained from vermicompost of olive cake by aeration as a bioremediation tool to enhance the biological degradation of pesticides.

MATERIALS AND METHODS

Soil and vermicompost

The soil was collected from an agriculture field in Granada (Spain). Before analysis, the soil was air dried and sieved to 4mm. The soil had a silty clay loam texture (34.0% clay, 56.0% silt and 10.0% sand) and it contained 44.1% $CaCO_3$. The soil field capacity was 33.2%.

Vermicompost of olive cake was obtained by vermicomposting a mixture of wet olive cake and manure in the ratio 4:1 by worms of Eisenia Andrei over 6 months and 2 months more for maturation and drying.

Pesticides

One insecticide: imidacloprid (99.9% purity); two herbicides: diuron (99.5% purity) and oxifluorfen (99.9% purity); and one fungicide: tebuconazole (99.5% purity) were selected as representative pesticides used in olive culture. Table 1 shows the environmental fate parameters of these pesticides. All the chemicals were supplied by Sigma-Aldrich (St. Louis, MO). These chemicals were dissolved in acetone separately as stock solutions. All other solvents and chemicals used were of HPLC grade.

Tea extraction

Vermicompost of olive cake and manure (4:1) (25 g) were weighted into a nylon bag with a pore size of 50 µm. A glass vessel was filled with 250 mL of distillated water and aerated two hours before adding the bag containing the vermicompost. Once the bag was introduced into the water, the sample was aerated at 20ºC in the dark for 24 hours. Four replicates were carried out. After this time, the bags were removed and the extracts or vermiteas were mixed and analyzed. Table 2 contains some vermitea properties. Biological oxygen demand (BOD), pH and conductivity were measured using the Cyberscan PCD 6500 meter (Thermo Scientific, Landsneer, Netherland). Dehydrogenase activity was determined using iodonitrotetrazolium formazan (INTF) as substrate, as described by García et al. [8]. Briefly, 1 mL of vermitea was added with 0.2 mL of distillated water and 0.2 mL of 0.4% INT (2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride) in distilled water for 20 h at 22 ºC in the dark. The INTF formed was extracted with 5 mL of a mixture of 1: 1.5 ethylene chloride: acetone by shaking vigorously for 2 min. Samples were centrifuged at 3500 rpm for 15 min. The INTF was measured in a

spectrophotometer at 490 nm. For the water soluble carbon determination, 0.5 mL of vermitea were added with 0.5 mL of distilled water, 1 mL of 1N $K_2Cr_2O_7$ and 2 mL of H_2SO_4. Sample was digested at 150ºC for 30 minutes and then measured in a spectrophotometer at 590 nm.

Two dosages of vermitea were used for the degradation study: i) a high dosage obtained as explained above from a 1:5 vemicompost: water ratio (w:v); and ii) a low dosage obtained by 1:2 (v:v) dilution of the vermitea at high dosage with distillated water.

Table 1. Chemical structure and selected physicochemical properties of the pesticides studied [7].

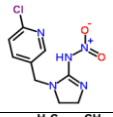
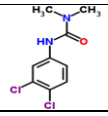
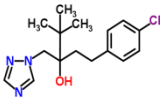
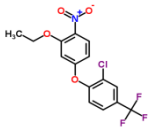
	Chemical Structure	Water Solubility (mg L^{-1})	Log K_{ow}
Imidacloprid		610.0	0.57
Diuron		36.40	2.85
Tebuconazole		36.00	3.70
Oxyfluorfen		0.12	4.47

Table 2. Physicochemical properties of vermitea obtained from 1:5 vermicompost: water ratio (w:v).

	BOD[a]	pH	Conductivity (mS cm^{-1})	Dehydrogenase activity (µg INTF $g^{-1}h^{-1}$)	WSC[b] (mg L^{-1})
Vermitea	7.98	7.56	3.94	76.48	1206.86

[a]BOD: biological oxygen demand.
[b]WSC: water soluble carbon

Degradation study

Soil samples (50 g) were spiked with 4 mL of an acetone solution containing a mixture of the pesticides studied. Final pesticide concentration in the soil was 5 µg g^{-1}. The samples were air dried in a fume hood to eliminate the solvent and homogenized by shaking in an end over end shaker for 5 minutes. The soil moisture content was adjusted to 80% of the soil field capacity adding ultra-pure water (S) or tea at low (STL) and high (STH) dosages. Samples were incubated in the dark at 20ºC for 60 days. Moisture content was maintained by regular additions of ultra-pure water. Immediately after pesticides application and at fixed intervals thereafter four samples from each treatment were removed and analyzed for pesticide concentration, dehydrogenase activity and dissolved organic carbon.

Extraction and analysis of pesticides

The extraction of pesticides from the soil samples was accomplished using the QuEChERS method. An aliquot of 7.58 g (equivalent to 6 g dry weight) of the homogenized sample was weighed into a 50 mL centrifuge tube and 3 mL of acetonitrile was added. The mixture was vortexed for 1 minute. Then, sample was added with 1.2 g of a mixture of salts which contained sodium citrate (15.4%), sodium hydrogencitrate sesquihydrate (7.7%), magnesium sulfate (61.5%) and sodium chloride (15.4%) (Agilent Technologies, Santa Clara, CA). The sample was immediately vortexed for 1 min to avoid agglomeration of salts and followed by centrifugation at 3000 rpm at 10ºC temperature for 5 min. An aliquot of 1 ml of the supernatant was diluted with 1mL of water. Recoveries of the extraction method ranged between 86% and 104%, depending on the pesticide. The pesticides were analyzed by HPLC-DAD (series 1100, Agilent Technologies, Santa Clara, CA) on a Zorbax RX-C8 column (5 µm, 2.1 x 150 mm) (Agilent Technologies, Santa Clara, CA) connected to an Eclipse XDB-C8 (5 µm, 2.1 x 12.5 mm) precolumn (Agilent Technologies, Santa Clara, CA). The mobile phase was acetonitrile and water adjusted to pH 3 with sulfuric acid. In order to get good separation of each analyte a solvent gradient was used from 20% to 70% of acetonitrile. The flow was set at 0.2 mL min^{-1}, the injection volume to 20 µL, the oven temperature to 40ºC, and the detector wavelengths to 270 nm for imidacloprid, to 210 nm for diuron and oxyfluorfen and to 215 nm for tebuconazole. Retention times were 8.1, 16.4, 20.5 and 25.7 min for imidacloprid, diuron, tebuconazole and oxyfluorfen, respectively.

Dehydrogenase activity

An aliquot of 0.5 g of soil sample was used for dehydrogenase activity determination. Dehydrogenase activity was carried out as described above using the method described by García et al. [8].

Water soluble carbon

Soil samples (2 g) were added with 10 mL of distillated water and extracted by agitation in a bath at 50 ºC for 1 h. After this time, samples were centrifuged at 3500 rpm for 10 min and filtered through filter paper. 1 mL of the extract containing the water soluble carbon was digested at 150ºC with $K_2Cr_2O_7$ and H_2SO_4 as explained above. Samples were measured in a spectrophotometer at 590 nm.

RESULTS

Degradation of the pesticides studied in soil and soil amended with vermitea followed a first order kinetics (Table 3). The coefficients of regression were greater than 0.83 for all soil samples and significant ($P< 0.05$), thus indicating that the assumption of first-order kinetic was acceptable.

In soil, the remaining concentration values of diuron and imidacloprid decreased faster than those of tebuconazole and oxyfluorfen as indicated by their higher degradation rate constant (K_d) and half-lives values (Table 3). At the end of the incubation time, the soil concentration was 49, 25, 74 and 66% of the initial amount of imidacloprid, diuron, tebuconazole and oxyfluorfen, respectively (Figure 1). The high hydrophobicity of tebuconazole and oxyfluorfen (Log $K_{ow}\geq 3.7$, Table 1) should render these chemicals more adsorbed to solid phase which protects them of being microbially degraded. In contrast, a significant fraction of diuron and imidacloprid, more water soluble (Table 1), may be present in the pore water where they can be degraded by soil microorganisms.

Table 3. Kinetic parameters obtained from the simple first order equation fit for pesticide residual concentration in soil (S) and soil amended with tea at low (STL) and high (STH) dosages.

		C_0 (%) ± sd	K_d (d^{-1})x10^2 ± sd[a]	R^2	$t_{1/2}$ (d)
S	Imidacloprid	82.2 ± 5.6	0.93 ± 0.26	0.88	77.01
	Diuron	82.9 ± 4.6	2.07 ± 0.30	0.97	34.66
	Tebuconazole	101.7 ± 5.1	0.51 ± 0.17	0.83	138.62
	Oxyfluorfen	106.6 ± 2.9	0.56 ± 0.09	0.95	86.64
STL	Imidacloprid	85.5 ± 4.3	1.11 ± 0.21	0.94	69.31
	Diuron	86.0 ± 2.9	2.04 ± 0.18	0.99	36.48
	Tebuconazole	104.9 ± 2.8	0.62 ± 0.09	0.96	115.52
	Oxyfluorfen	107.0 ± 3.0	0.76 ± 0.10	0.97	99.01
STH	Imidacloprid	82.9 ± 5.6	0.91 ± 0.29	0.85	86.64
	Diuron	84.5 ± 8.1	1.61 ± 0.46	0.88	53.32
	Tebuconazole	103.3 ± 4.8	0.41 ± 0.16	0.78	173.28
	Oxyfluorfen	105.2 ± 4.6	0.75 ± 0.16	0.93	138.62

[a]Standard deviation (n=4)

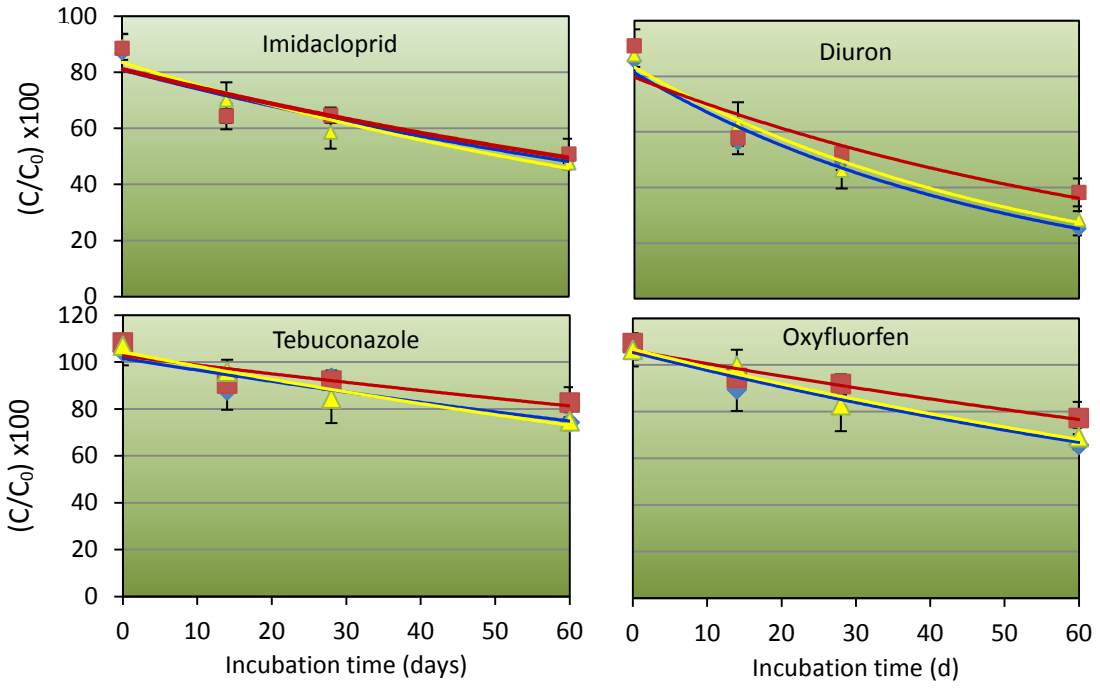


Figure 1. Pesticide degradation in soil (◆) and soil amended with vermitea at low (△) and high (■) dosages. Experimental data were fitted to a first order equation. The vertical lines represent the standard deviation in each sample (n=4).

The addition of vermitea to soil did not increase its overall ability to degrade the four pesticides considered in this study. Thus, at the end of the incubation time, the concentration of the different pesticides in STL was not significantly different ($P > 0.05$) from that in S (Figure 1). Addition to soil of vermitea at high dosage did not have any effect on imidacloprid and tebuconazole degradation (Figure 1). However, at the end of the incubation time, the remaining concentrations in soil of diuron and oxyfluorfen were significantly higher ($P \leq 0.04$) in STH than in S (Figure 1). These results suggest an inhibitory effect of vermitea on the microbial activity of the soil.

Dehydrogenase activity has been used as indicator to estimate the total microbial activity of soil [9, 10]. The highest dehydrogenase activity was observed at 15 days of incubation in non-amended and soil amended with vermitea (Figure 2). STH presented the highest dehydrogenase activity at this time as it was expected because its highest microbial mass and higher water soluble carbon content (Figure 3). However, after 15 days of incubation, dehydrogenase activity in STH decreased markedly regard to STL (Figure 2) indicating that high concentration of vermitea may have a negative effect on soil microorganisms as indicated above. Despite the higher microbial activity of soil amended with vermitea regard to non-amended soil during the first month of incubation, in general, pesticide degradation was not significantly different between treatments suggesting that the vermitea´s microorganisms may have preferred the more easily degradable organic compounds of the vermitea as a carbon and or nitrogen source instead the pesticides. Other authors have considered this hypothesis previously for olive cake and different organic amendments [11-13].

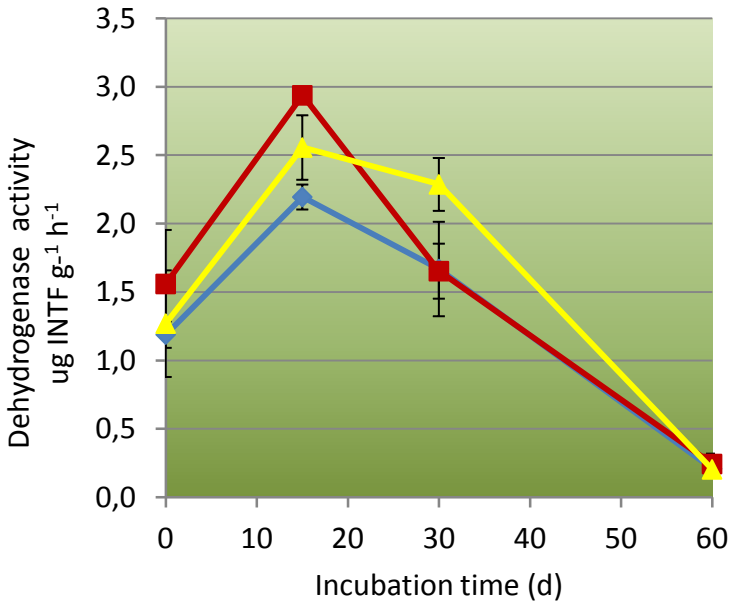


Figure 2. Dehydrogenase activity in soil (◆) and soil amended with vermitea at low (△) and high (■) dosages. The vertical lines represent the standard deviation in each sample (n=4).

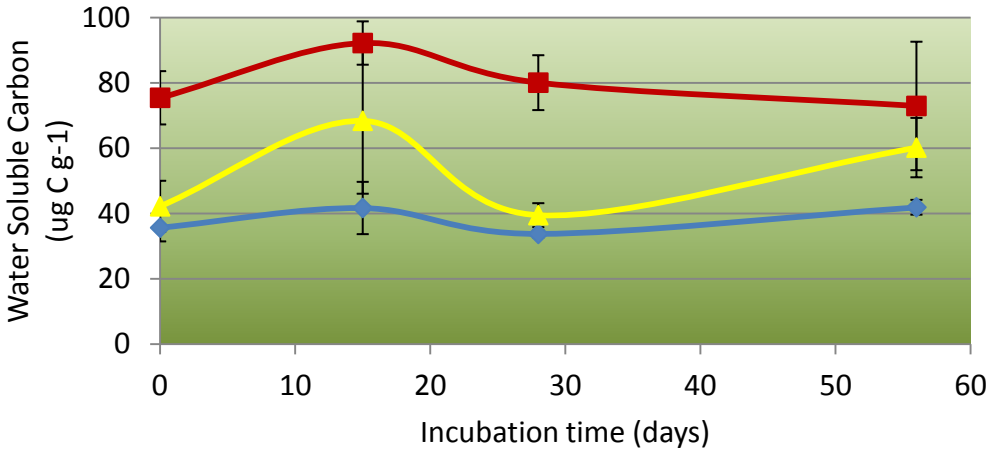


Figure 3. Water soluble carbon in soil (◆) and soil amended with vermitea at low (△) and high (■) dosages. The vertical lines represent the standard deviation in each sample (n=4).

CONCLUSIONS

1. **Imidacloprid and diuron were the pesticides more degraded. Degradation of tebuconazole and oxyfluorfen, more hydrophobic compounds, is limited by sorption to soil particles.**
2. **Addition of vermitea to soil did not modify the soil degradation capacity for pesticides.**
3. **The addition of vermitea to soil stimulated general microbial population activity without concurrent increases in pesticide degradation, suggesting that the specific microbial populations responsible for degrading the pesticide were not stimulated or that microorganisms prefer more labile compounds present in vermitea as nitrogen and carbon sources rather than pesticides.**

ACKNOWLEDGEMENTS

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MY OWN IDEAS

Laura León Rejón

This experience has been very pleasant, because I like everything related to nature and this has been very interesting. At first, the entire hypothesis seemed to me reasonable, if the tea removes contaminants from people, why could not it do the same from an agricultural soil? After doing the experiment with different doses of vermitea we have found that addition of high dosage of vermitea to soil may have a negative effect on microbial activity. If I were asked about defining Piiisa project with a single word I will have to say: RESULTS. Although the project has not gone as expected, we have found great things about how the soil affects the pesticide and its impact. This has been an unforgettable experience and, of course, everyone should experience it because it really worth it.

Esta experiencia ha sido muy fructífera, ya que a mí me gusta mucho todo lo relacionado con la naturaleza y éste ha sido un proyecto muy interesante. En un principio, todas las hipótesis me parecían razonables y poco a poco el conocimiento llevó a la hipótesis de que si el té elimina sustancias contaminantes de las personas, ¿por qué no se podía hacer con el suelo?. Después de hacer el experimento con distintas dosis de té hemos descubierto que éste, en dosis altas, puede tener un efecto negativo para la actividad microbiana.

Si alguna vez me preguntasen que cómo definiría PIIISA en una sola palabra, ésta sería: resultados. Aunque nuestro proyecto no haya salido como esperábamos hemos descubierto gran cantidad de cosas sobre los plaguicidas y la tierra. Ésta es una experiencia inolvidable que debería experimentar todo el mundo.

Belén García Trigueros

The existence of pollution in the environment and its consequences for people´s health and ecosystems is not something not everybody is aware of. The aim of this project is to test the efficiency of vermicompost “teas” made of organic residues in order to obtain a faster degradation of pesticides used in fields for cultivation.

I decided to choose this project because the idea of being able to provide new environmentally friendly techniques seemed very interesting to me, especially when it comes to two vital elements such as soil and the water.

Despite not achieving our initial expectative and success in this work at first, it has given me the opportunity to participate fully into a real scientific investigation process. Surely, scientists’ efforts will eventually lead to the achievement of new techniques which will make our intended objective a reality.

I knew about PIIISA through my high school center and our teachers encouraged us to participate.

My experience has been truly satisfactory, though I have worked in areas which are not exactly the ones I would like to keep on working in the future. I think there are very different projects that offer students the possibility of learning about the scientific method.

La presencia de contaminantes en el medio ambiente y su repercusión en la salud humana y los ecosistemas es una realidad que no suele tenerse en cuenta. El objetivo del proyecto que

hemos desarrollado es determinar la eficacia de "tés" de vermicompost procedente de residuos agrícolas orgánicos para una degradación más rápida de los plaguicidas utilizados en los suelos de cultivo.

Elegí este proyecto porque me pareció muy interesante la idea de poder contribuir a desarrollar técnicas que cuiden el medio y recursos tan importantes como el suelo y el agua.

Aunque no hemos obtenido los resultados esperados en un principio, el proyecto me ha dado la oportunidad de sumergirme de lleno en la investigación científica. Estoy segura de que con el tiempo y el esfuerzo de investigadores se obtendrán técnicas que cumplan con el objetivo que intentábamos alcanzar.

Conocí PIIISA a través de mi centro educativo donde los profesores nos animaron a participar.

Mi experiencia al desarrollar este proyecto ha sido muy satisfactoria, a pesar de que trataba materias que no me gustaría seguir trabajando en un futuro. Creo que hay una gran diversidad de proyectos que ofrecen a los estudiantes la posibilidad de acercarse a la investigación.

María Rosales Reyes

The PIIISA project has meant to me a great opportunity to know how scientists make research and to understand why research is useful for our society. This project is a great advantage for those who want to pursue any field of research.

To be honest I considered this project at the last place because I prefer other projects more related to biomedicine or research related to disease and human body but also it has helped me to learn and this project has been as interesting as it could have been any other related to what I want to study.

Although we have not obtained the results expected at the beginning, it was a project of great interest to which I have shared time and desire with great colleagues which have been interested in our work a lot. I would truly like to repeat this experience and I think Piiisa project is an excellent and with great future project since it gives a great opportunity to somehow young people feel like scientists.

El proyecto PIIISA ha significado para mí una gran oportunidad para poder ver como se investiga y para lo que sirve la investigación. Este proyecto es una gran ventaja para todos aquellos que quieren dedicarse a cualquier campo de investigación.

Siendo sincera cogí este proyecto de los últimos porque mi dirección va más hacia la biomedicina o investigaciones relaciones con enfermedades y el cuerpo humano pero también me ha servido este proyecto para aprender y ha sido igual de interesante que podría haber sido cualquier otro relacionado con lo que quiero estudiar.

Aunque en nuestro proyecto no hemos obtenido los resultados que pretendíamos ha sido un proyecto de gran interés en el cual he compartido tiempo y ganas con unos grandes compañeros los cuales se han volcado mucho en nuestro trabajo. Me encantaría repetir esta experiencia y veo muy bien y con gran futuro a este proyecto ya que da la gran oportunidad de alguna manera de sentirse como un científico.

Carlos Soto Paniza

In this project, we have studied how pesticides are biologically degraded in the soil by adding tea obtained from vermicompost (vermitea) which we have used to provide greater resistance of plants to diseases.

To check the effectiveness of the vermitea we have tested the degradation of pesticides in soil of three different ways:

- Soil added with vermitea at high dosage.
- Soil added with vermitea at low dosage.
- Soil added with water.

In this project we have learned to use scientific tools which I have not known so far, we have to learn how to weight accurately and precisely, to measure the pH and various other techniques ...

I decided to participate in the Piiisa project to try to understand how scientists work in a laboratory and also to know the instrument and other tools used in research and the science procedure. I found the Piiisa project very interesting because I have had the opportunity to know new people and colleagues. Although, I have to complain about the number of mandatory visits. I think we need more visits in order to perform the work with more time and dedication.

En este proyecto hemos estudiado como degradar biológicamente los plaguicidas en el suelo por adición de tés de vermicompost los cuales hemos empleado para dotar de mayor resistencia a las plantas frente a enfermedades.

Para comprobar la eficacia del vermité hemos probado la degradación de plaguicidas en suelo de tres maneras diferentes:

- Suelo adicionado con vermite a dosis alta.

- Suelo adicionado con vermite a dosis baja.

- Suelo adicionado con agua.

En este proyecto hemos aprendido a utilizar herramientas científicas no conocidas para mi hasta el momento, hemos a prendido a pesar de manera exacta y precisa a medir el pH y diversas cosas más...

Decidí entrar en el proyecto Piiisa para intentar entender más como es el trabajo en un laboratorio y conocer aparatos herramientas e infinidad de cosas que se pueden hacer en él, y de paso observar cómo sería una investigación importante con sus resultados y procedimientos. Me ha parecido muy interesante haber conocido este proyecto por haber conocido nuevas personas y compañeros con los que he trabajado el proyecto. Aunque hay una pega, que es la falta de más visitas obligatorias para poder realizar los trabajos con más tiempo y dedicación para la los alumnos que están en este proyecto.

Celia Cámara Pérez-Vela

I will start saying that the PIIISA project is an interesting project that I personally had not heard until 2014. I am very proud to have the possibility of participating in something like this, and have met all the people who have helped me with it.

I think the organization of this project is pretty good, but I also think it could be better with the ideas as follows:

- Increase the number of visits required, because with only three of these visits students with greater difficulty (whether economic or by the distance of their homes) will be absent in meetings to advance the project they've been assigned.
- If the period of the investigations was longer it would be really great for doing more things on each research.

On the other hand, the willpower and the desire of students to engage in this project are essential. The research voluntarily chosen by the students would significantly increase their involvement in that project.

Talking about my investigation group, we have reached interesting results, though it was not what we wanted to get, we have taken several positive conclusions from it, and this enables guided by another way to have what we want to achieve, which is the elimination of pesticides in soil. I have to say that I have learned a lot of it, and it would be excited to repeat something like this. The only problem is that I live far away from the site where we have the visits, so I could not contribute all that I wanted. Because of that I think the number of visits required should increase.

Overall, I think the project has been a good idea for development work and commitment to young students, and I hope it to continue being like this for a long time.

Empezaré diciendo que el proyecto PIIISA es un interesante proyecto del que personalmente no había oído hablar hasta 2014. Estoy muy orgullosa de tener la posibilidad de participar en algo así, y de haber conocido a personas que me ayudaron con ello.

Creo que la organización del proyecto está bastante bien, pero también creo que podría mejorarse con las siguientes ideas:

Incrementar el número de "visitas obligatorias", ya que únicamente con tres de estas visitas, los estudiantes con mayor dificultad (ya sea económica o por la distancia de sus casas) se ausentarán en las demás visitas voluntarias para avanzar en el proyecto al que se les haya asignado.

Si el periodo de investigación fuera más largo, sería genial para investigar más en cada proyecto.

Por otro lado, la fuerza de voluntad y el deseo de los estudiantes de involucrarse en este proyecto son esenciales. La elección voluntaria de la investigación a realizar por cada estudiante, supondría una involucración significativa de éste en el proyecto.

Hablando sobre mi grupo de investigación, hemos logrado interesantes resultados, a pesar de que no era lo que queríamos conseguir en un principio, hemos obtenido de ello conclusiones positivas, lo que nos permite guiarnos por otro lado para llegar a lo que queremos lograr, que es la eliminación de plaguicidas en suelos. Tengo que decir que he aprendido mucho de ello, y sería emocionante repetir algo así. El único problema es que vivo lejos del sitio en el que se realizan las visitas, así que no he podido aportar todo lo que habría querido. Es por eso que creo que el número de visitas obligatorias debería aumentar.

A pesar de todo, creo que el proyecto ha sido una buena idea para el desarrollo en trabajo y compromiso de los jóvenes estudiantes, y espero que siga siendo así durante mucho tiempo.