ERDC/EL MP-20-10

US Army Corps
of Engineers®
Engineer Research and
Development Center

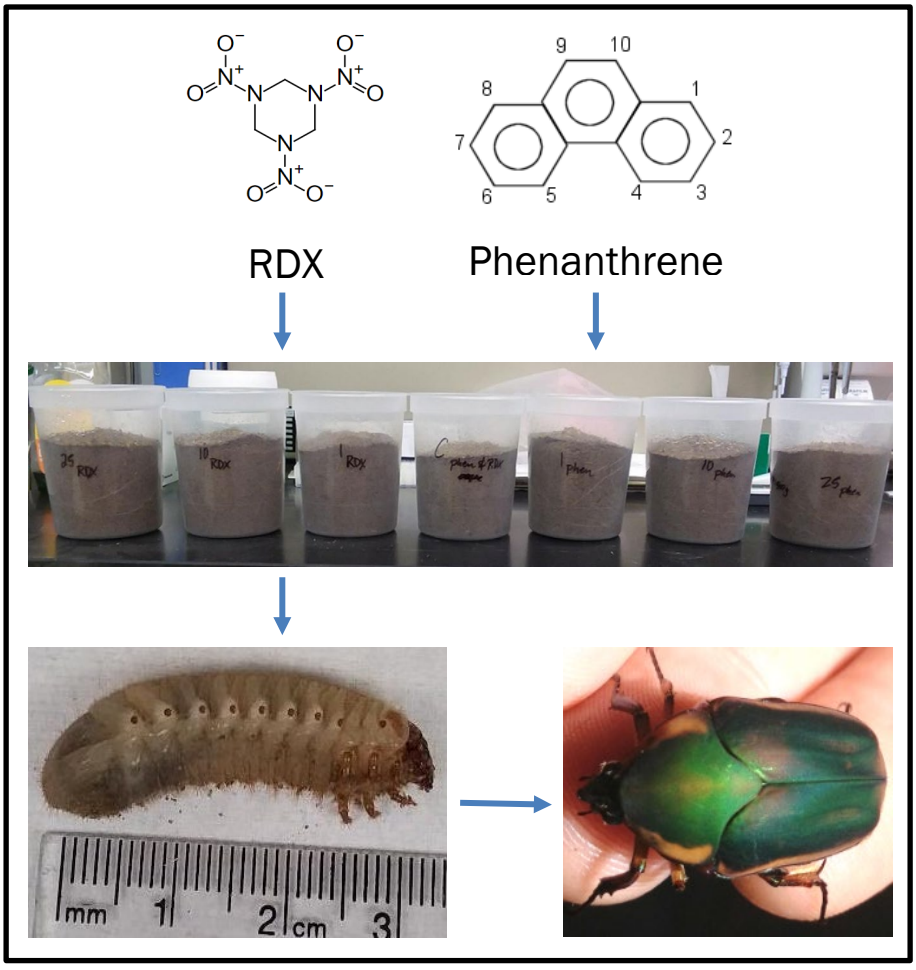
ERDC
ENGINEER RESEARCH & DEVELOPMENT CENTER

Environmental Quality Program

Response of the Green June Beetle and its Gut Microbiome to RDX and Phenanthrene

Carina M. Jung, Matthew Carr, Eric Fleischman, and
Chandler J. Roesch

November 2020



Environmental Laboratory

The U.S. Army Engineer Research and Development Center (ERDC) solves the nation's toughest engineering and environmental challenges. ERDC develops innovative solutions in civil and military engineering, geospatial sciences, water resources, and environmental sciences for the Army, the Department of Defense, civilian agencies, and our nation's public good. Find out more at www.erdc.usace.army.mil.

To search for other technical reports published by ERDC, visit the ERDC online library at https://erdclibrary.on.worldcat.org/discovery.

Environmental Quality Program

ERDC/EL MP-20-10
November 2020

Response of the green June beetle and its gut microbiome to RDX and phenanthrene

Carina M. Jung, Matthew Carr, and Chandler J. Roesch

Environmental Laboratory
US Army Engineer Research and Development Center
3909 Halls Ferry Road
Vicksburg, MS 39180-6199

Eric Fleischman

ThermoFisher Scientific
Eugene, OR 97402

Final report

Prepared for US Army Corps of Engineers
Washington, DC 20314-1000

Under ERDC – EQI - 6.1 Military Direct Program, Project 18-040, “Dung balls and grass roots: Nature’s tiny bioremediation factories”

Abstract

Green June beetles are a cosmopolitan pest in the United States. Adults are voracious consumers of tree and vine fruit, while their larvae can damage and inadvertently consume root systems, particularly those of grasses, as they move through the soil and forage for detritus. Larvae ingest and process large volumes of soil while in the process of feeding. Due to their intimate contact with the soil it was hypothesized that soil contaminants that are known animal toxins would perturb the larval and affect their overall health and survival. Studies of this kind are important contributions to the development of new model organisms and our understanding of interactions between the environment, contaminants, gut microbiome, and animal development, health, and survival. It is important to continue to develop relevant model organisms for monitoring toxicity as regulations for working with vertebrates becomes more prohibitive. In this study green June beetle larvae were exposed to RDX and phenanthrene throughout their entire soil-bound development, starting within the first few days of hatching through to their emergence as adults. The overall findings included that even at high concentrations, RDX and phenanthrene (25 ppm) exerted no significant effect on body weight or survival. Also, there was little apparent effect of RDX and phenanthrene on the bacterial microbiome, and no statistical association with measurable health effects. Nevertheless, the green June beetle is an interesting model for soil toxicity experiments in the future as is it easy to collect, house, and handle.

Contents

Figures and Tables

Figures

Tables

Preface

This study was conducted for the US Army Corps of Engineers, Engineer Research and Development Center (USACE ERDC) under ERDC – EQI - 6.1 Military Direct Program Basic Research Program initiated in FY2018, as Project 18-040, "Dung balls and grass roots: Nature's tiny bioremediation factories"

The work was performed by the Environmental Processes Branch (EPP), Environmental Processes and Engineering Division (EPED), US Army Engineer Research and Development Center, Environmental Laboratory (ERDC-EL). At the time of publication, Dr. Brandon Lafferty was Branch Chief, EPP; and Mr. Warren Lorentz was Division Chief. The Deputy Director of ERDC-EL was Dr. Jack Davis and the Director was Dr. Edmund Russo.

This paper was originally published in the International Journal of Environmental Science and Technology on 10 October 2020.

COL Teresa Schlosser was the Commander of ERDC, and Dr. David Pittman was the ERDC Director.

1 Introduction

Adult green June beetles (*Cotinis nitida*) (Figure 1) are voracious consumers of tree and vine fruit. The larvae of green June beetles (GJBs), which persist in the larval phase for 8 to 18 months, can disrupt and inadvertently consume entire root systems as they move through the soil foraging for detritous. The larvae, often identified as "white grubs" or turf grubs", are well-known major pest of golf courses and large turf areas (USEPA 2003). Larvae ingest and process large volumes of soil while in the process of feeding. Due to their intimate contact with the soil it was hypothesized that soil contaminants that are known animal toxins would perturb the microbiome of developing larvae and affect their overall health and survival.

Figure 1. Adult green June beetle (*Cotinis nitida*).

GJBs are known to harbor fermentative yeasts in their gut microbiome (Vishniac and Johnson 1990). These yeasts are responsible for the breakdown of simple fruit sugars. The gut microbiome develops upon emergence from the pupal stage into adulthood through inoculation by their environment. The larval microbiome has not been studied with much interest but it is expected to be markedly different from the adults as larval diet consists of soil organics, plant roots, detritus, microfungi, and other scavenged topsoil and soil surface materials rather than simple fruit sugars (Shukla et al. 2016). There is a paucity of information regarding the effects of contaminants on soil dwelling insects or their gut microbiomes. As a means to fill this knowledge gap and understand more fully how the associated gut bacteria may be effected or help modulate the effects of potentially toxic compounds, a bacterial population survey was conducted via 16S rRNA high-throughput sequencing (Illumina MiSeq) in both the larval and adult life stages. Of equal importance is how the gut microflora of

these life stages differ and are differentially affected by environmental stressors.

Two soil contaminants commonly found on military ranges and installations were introduced to the soil housing larvae at three different concentrations. RDX (royal demolition explosive; 1,3,5-trinitro-1,3,5,-triazacyclohexane) is both a legacy and currently used explosive worldwide and phenanthrene is a common contaminant found most heavily around industrial sites and coal burning power plants (USEPA 2019). There is no toxicity information on either of these compounds for June beetles, or indeed many insects. Information gathered regarding toxicity to the soil dwelling life stage of this insect and possible effects on the gut microbiome would be of interest to toxicologists and environmentalists at large.

2 Methods and Materials

2.1 Collection and housing of beetles

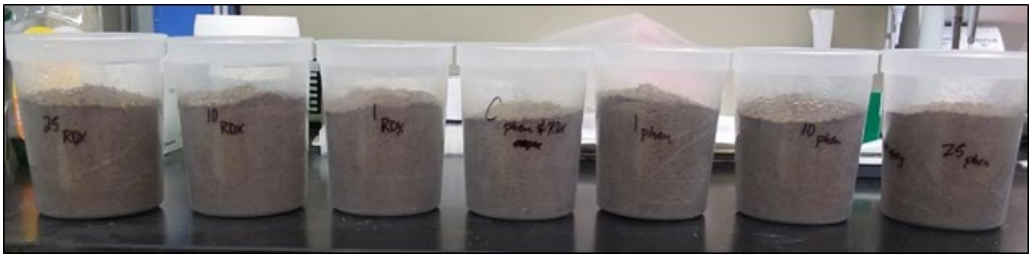
GJB adults (n=80) were trapped in agricultural fields at the University of Arkansas Agricultural Experiment Station in Fayetteville, AR using modified vane traps. GJBs were housed for breeding at the Engineer Research and Development Center in Vicksburg, MS following of Johnson, et al 2009. Briefly, adults were housed in a 30 gallon food grade plastic can with PVC pipes extending 2 feet beyond the opening and a household door screen cover to produce a tented "flight-space" (Figure 2). Soil substrate was added to 1 foot depth using clean soil from ERDC, organic topsoil, and sand (6:3:1). Adults were fed on a diversity of organic, store-bought mangoes, Pink Lady apples (with outer peel removed), and grapes. Eggs were collected at least weekly and placed in 1 gallon buckets containing 1/3 volume of the same soil mixture with organic alfalfa meal (Epsoma, Millville, NJ) added to the surface. Summer room temperature with filtered natural light and a 12:12 light cycle was provided along with daily spray of water (Howe and Campbell 1953; Johnson 2009). Eggs were collected and hatched to larvae for toxicology studies.

Figure 2. GJB adult rearing chamber.

2.2 Experimental setup

Ten larvae per exposure group for each beetle were exposed to 500 g soil dosed with final concentrations of 0 ppm (mg kg^{-1}), 1 ppm, 10 ppm, and 25 ppm RDX or phenanthrene (Figure 3) in 1 qt deli cups. These June beetle larvae "exposure groups" were added to prepared soil at day 1 of hatching. Organic alfalfa meal was added food source to the surface of the soil. Larvae were evaluated for overall growth and health (weight, size, and survival) through to the 3rd instar (>3 months) in each treatment. Larvae and soil were sampled (as described in section 2.3) at the start of the experiment for baseline microbial community characterizations and contaminant concentration measurements (as described in section 2.4). At the second instar, 5 larvae were sacrificed for microbiome analysis. Remaining larvae were allowed to continue development and were sacrificed upon emergence into adults for microbiome analysis.

Figure 3. Toxicological experimental setup. Contaminated and control soils housed n=10 larvae from hatching to adult emergence.



2.3 Bacterial communities identified

Larvae (n=5) at 0 and 38 days were frozen at -20°C for 5 min, surface sterilized in 95% ethanol, the abdomen was liberated from the thorax (Figure 4) of each larvae and the gut contents were extruded and homogenized by vigorous vortexing. A portion of the homogenate (0.1 g) was extracted for DNA. Adults were sampled by dissection and extrusion of the gut contents in the same manner as larvae. The DNA from the gut material was extracted, PCR amplified, and run through next-generation sequencing with the Illumina MiSeq platform (Illumina, Inc., San Diego, CA) as described elsewhere (Caporaso et al. 2010; Caporaso et al. 2012; Caporaso et al. 2011). Briefly, DNA was extracted via a MoBio PowerSoil kit following the manufacturer's instructions and the resultant DNA was amplified with uniquely barcoded primers specifically designed for 16S rRNA bacterial se-

quencing (515-806 bp region of the 16S gene) (Caporaso et al. 2012). Amplicons were combined and normalized to 15 pmol and further combined with 10% PhiX control according to Illumina MiSeq instructions. The QIIME (Quantitative Insights Into Microbial Ecology) bioinformatics pipeline (http://qiime.org/) was used to analyze the sequencing data. Principal coordinates analysis (PCoA) was conducted using pairwise distances between bacterial communities (UniFrac distances) to determine if sample categories contained significantly different microbial communities. Relative abundances of bacterial community composition were assessed at the 1% abundance threshold and significant differences in communities between samples were assessed by Kruskal Wallis using a false discovery rate (FDR) P-value of 0.05.

Figure 4. Second instar larvae at 38 days. The red arrow indicated the point of where the abdomen was liberated from the thorax.

2.4 Contaminant profiles

The remaining gut homogenate material was weighed and prepared for chemical extraction along with grab samples of soil. Phenanthrene and RDX were extracted using procedures for solid material (feces, tissue, or soil) outlined previously (Lu and Lu 2015; Sun et al. 2015; Yuan 2010). Briefly, the soil or gut contents were sonicated for 30 min with 5X volume of dichloromethane and extracted twice more with the same volume. The solvent was evaporated under a N_2 stream and the sample was resuspended in either hexane (GC-MS) or methanol (HPLC). Analyses were conducted using the following modified EPA 8330 method (Crocker et al. 2006; Jung et al. 2011): Phenanthrene was analyzed by HPLC and confirmed by GC-MS following HPLC (Sun et al. 2015) and GC-MS (Lu and Lu 2015; Lundin et al. 2015; Yuan 2010) methods and RDX was analyzed by HPLC (Crocker et al. 2005; Jung et al. 2011). The GC-MS method was run on an HP 5890 GC with an HP 5973 MS detector using a 30M × 250µm × 0.25µm Perkin Elite 5MS column. One µl of analyte was injected with a 0.6:1 split with a flow rate of 3.6 ml min^{-1}, with the oven set to an initial

temperature ramp of 15°C min^{-1} from 40°C to 100°C followed by a 1 min hold, then the same ramp to 225°C and a 4 min hold. The final temperature ramp of 10°C min^{-1} to 325°C was followed by a 4 min hold. The aux temperature was set to 325°C, the source was set to 230°C, and the MS-Quad was set to 150°C. The HPLC method was used for both chemicals and was run on an Agilent 1260 Infinity with a DAD detector set at 220 nm (Phenanthrene derivatives) and 234 nm (RDX and phenanthrene) and an Eclipse Plus C18 (4.6×100 mm×3.5 µm) column held at 38°C with 25 µl sample injection and a 1.2 ml min^{-1} flow rate. The mobile phases were ramped after a 5 min hold from 32:68 methanol:water to 90:10 over 7 min.

3 Results and Discussion

There were no statistically significant differences in survival (Table 1 and Figure 5) or body weight (Figure 6) between controls and exposure groups for either RDX or phenanthrene ($p>0.05$). Neither RDX nor phenanthrene were degraded by the larvae by the 38 day time point (Figure 7). No degradation of RDX was observed in the soil of controls or larvae

and roughly 10% of the RDX was recovered from the larval extracts (Figure 7A). Phenanthrene is a notoriously difficult compound to recover from the soil. Extended extractions are typically needed. A modified extraction method was used to process both RDX and phenanthrene samples simultaneously. Although highly successful for RDX, this method was substandard for complete extract of phenanthrene. However, the "no larvae control" soil at 25 ppm shows the same concentration as the larvae 25 ppm group and no breakdown products were detected (Figure 7B). Phenanthrene appeared to be readily extracted from the larvae as around 70% of the 25 ppm phenanthrene was recovered from the larvae with no breakdown products observed.

Table 1. Percent survival of larvae at the conclusion of the toxicity experiment (242 days).

	% survival at 242 days
Control	57.14
Phen 1	57.14
Phen 10	28.57
Phen 25	57.14
RDX 1	71.43
RDX 10	42.86
RDX 25	42.86

Figure 5. Percent survival of larvae at the conclusion of the toxicity experiment (242 days).

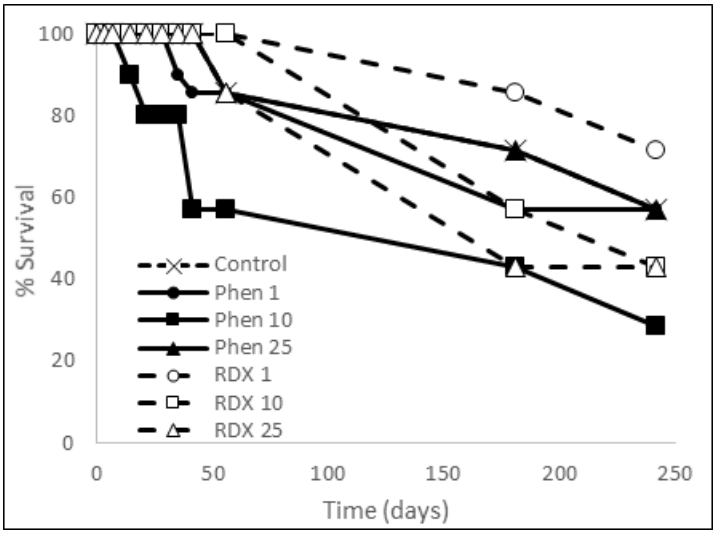


Figure 6. Average body weight of larvae during the toxicity experiment (242 days). Error bars represent the standard deviation (n = 3-10).

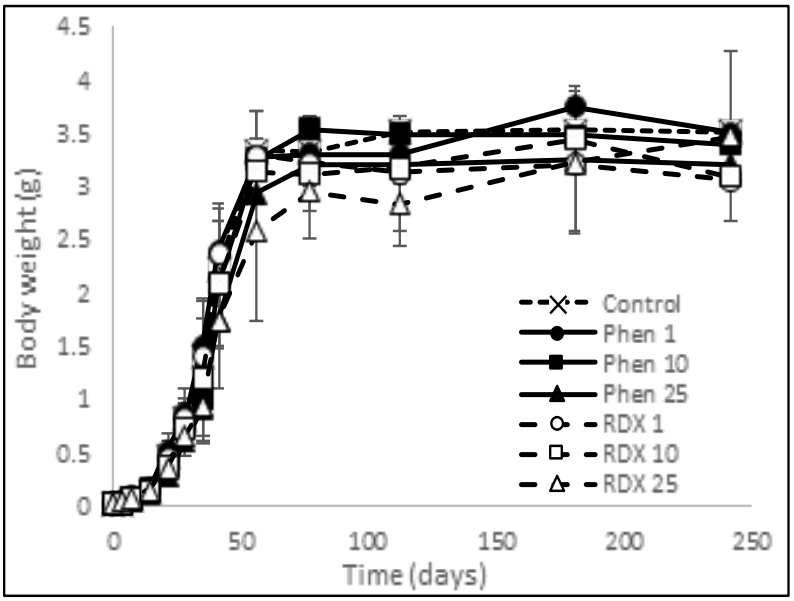
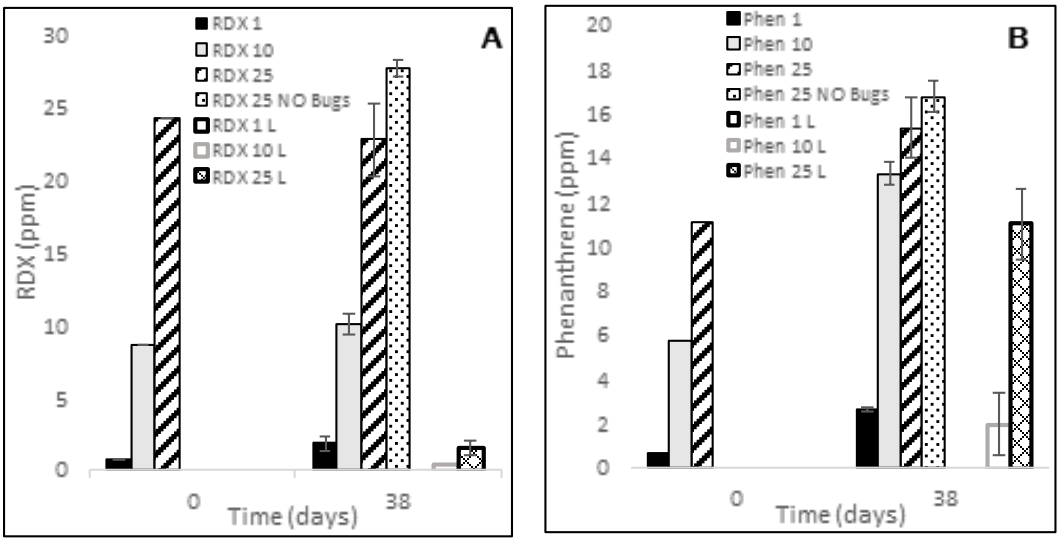
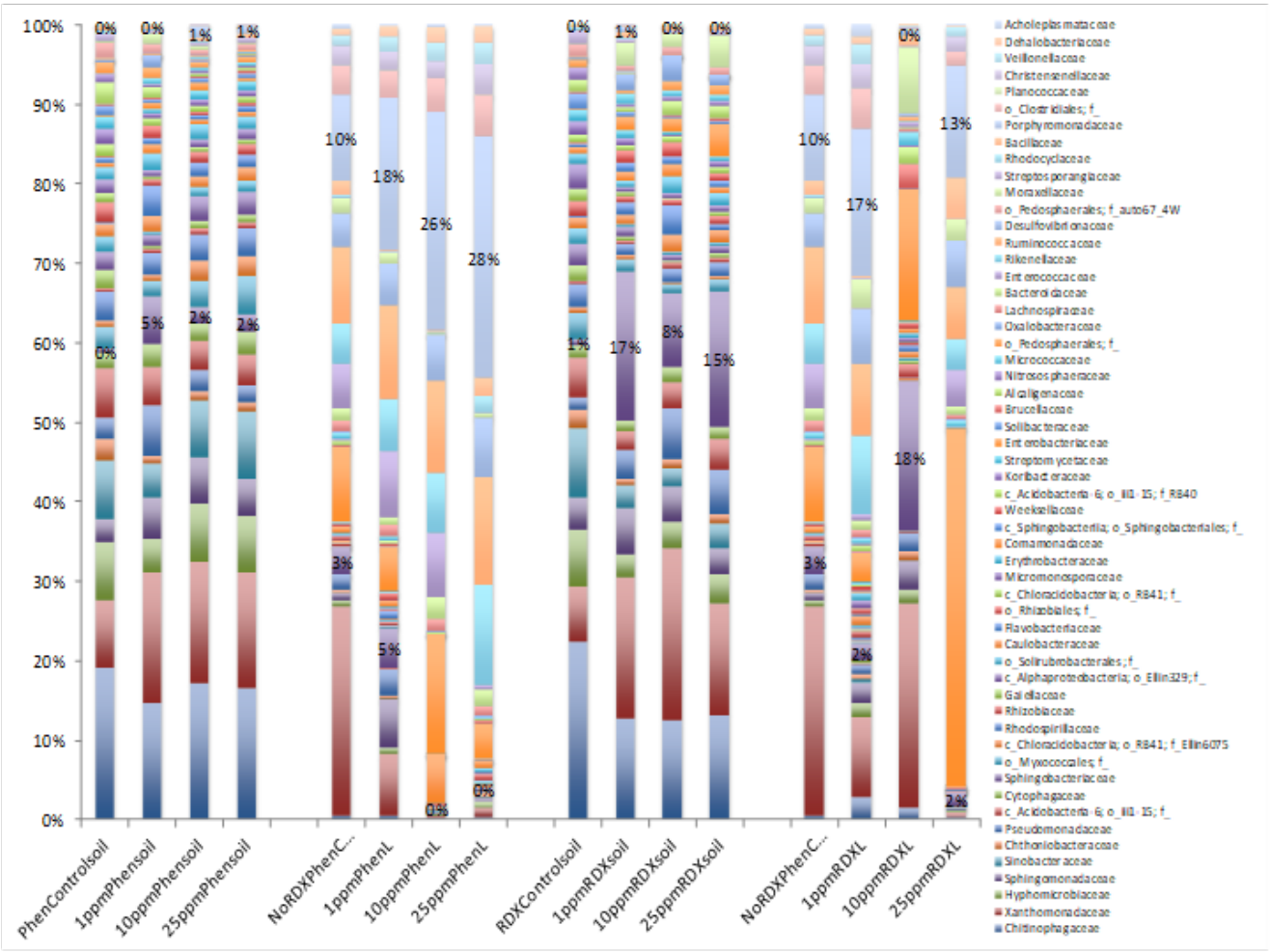


Figure 7. Concentration of RDX (A) and Phenanthrene (B) in the treatment soils spiked at 1, 10, or 25 ppm and larvae (L) from day 0 to day 38 as compared to the control (no bugs) soils.



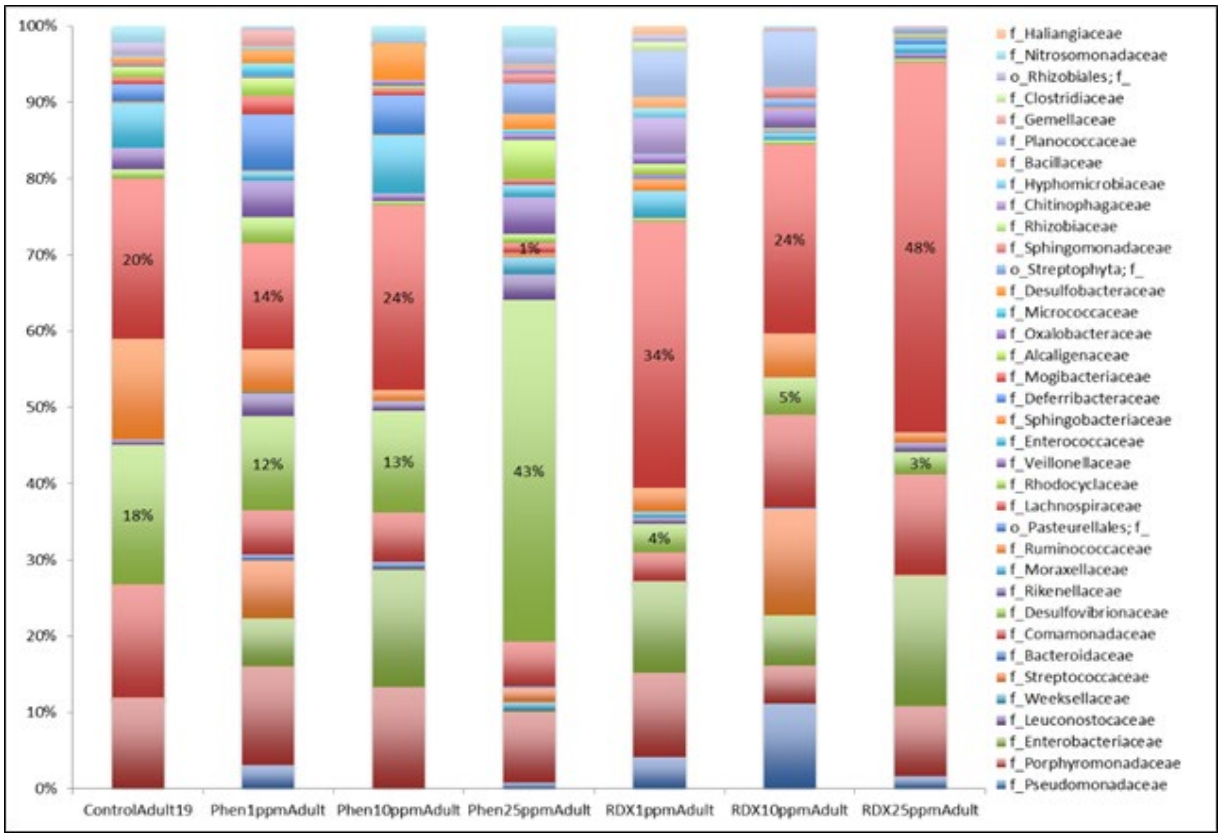
Bacterial community surveys conducted on the larvae eviscerates in the RDX and phenanthrene toxicity study were compared to respective soil bacterial communities (Figure 8). An increase in the Sphingomonad population was seen in the RDX spiked soils but was not transferred to the larval gut microbiome, with the exception of the 10 ppm RDX larval group. Jayamani, et al. (2013) noted RDX degradation in previously unexposed agricultural soils by organisms in the Sphingomonadaceae family family (Jayamani et al. 2013). Although there was no demonstrable deg-radation of RDX, these bacteria may be better suited to withstand the toxic effects of RDX than other bacteria. Overall, there were no predicable patterns or trends seen in the RDX treatments. The phenanthrene spiked soils looked very similar to the control soil. Interestingly, the larvae ex-hibited an increasing trend toward the proliferation of the Porphyromon-adaceae family with increased phenanthrene concentration. Although this points to some perturbation, the significance is unclear. Members of the Porphyromonadaceae are generally found in the oral cavity of mam-mals and when found in abundance in the gut they are an indicator of in-testinal dysregulation (Olsena and Yamazakib 2019). This could point to a negative effect of increasing levels of phenanthrene on larval gut micro-biome, but additional experimental effort would be required to arrive at truly robust hypotheses in this regard.

Figure 8. Phylogenetic survey at the family level of soil and larvae (L) gut microbiomes exposed to either RDX or phenanthrene (Phen), or unexposed (control/NoRDXPhen) for 38 days during the toxicity study. The Sphingomonadaceae are denoted by the purple bars with data labels and the Porphyromonadaceae by the light blue bars with data labels.



When comparing the gut bacterial communities of adults that emerged after developing in contaminated soils (Figure 9) there were no significant differences between any of the treatment groups (Kruskal Wallis; FDR - $p > 0.09$). All treatment groups also exhibited similar communities to that of the control group. This points to a lack of larger toxicity effects of either RDX or phenanthrene on the gut microbiome, as the larvae metamorphosed into adults despite being exposed to these contaminants throughout their entire growth cycle.

Figure 9. Phylogenetic survey at the family level of emergent adult GJB gut microbiomes exposed to either RDX or phenanthrene (Phen), or unexposed (control) for the duration of the toxicity study. The Lachnospiraceae are denoted by the red bars with data labels and the Desulfovibrionaceae by the light blue bars with data labels.



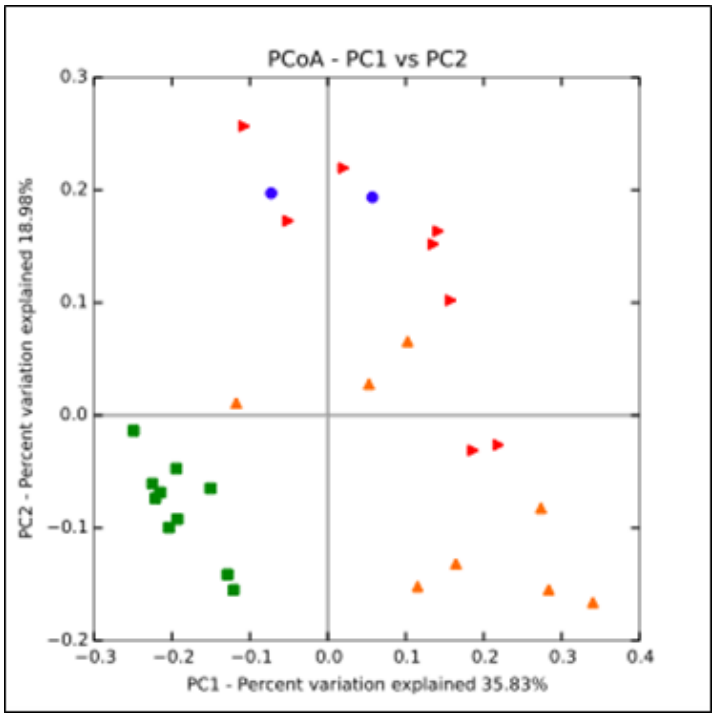
Due to the low sample numbers, statistics for the adult microbiome were remedial (Table 2). However, there were noteworthy differences in the Lachnospiraceae family as it was greatly diminished in the phenanthrene 25 ppm treatment. These are a common gut bacteria that ferment plant polysaccharides. There was only one survivor in this treatment so conclusions are difficult to make. However, one might speculate that perhaps losing this population of Lachnospiraceae led to poor health and caused other members of this group difficulties through larval development and/or pupation. Another noteworthy difference was that in the phenanthrene 25 ppm treatment again, there was a dramatic increase in the Desulfovibrionaceae family. In mice this particular shift of a lower prevalence of Lachnospiraceae and a higher prevalence of Desulfovibrionaceae are seen in high animal-fat diets (Just et al. 2018). This is perplexing, but gives rise to a tantalizing theory that perhaps the lone survivor cannibalized its group mates.

Table 2. Percent survival and completion of metamorphosis into adult at the conclusion of the toxicity study.

Adult Survival (%)	
Control	40
Phen 1	60
Phen 10	20
Phen 25	20
RDX 1	80
RDX 10	60
RDX 25	60

Weighted UniFrac principal coordinates analysis (PCoA) plots were performed on all the samples from this study as a point of curiosity (Figure 10). Not unexpectedly, the most pronounced separation of samples was that done by sample source. As easily seen in the bar charts, the soil samples are markedly different from all other sample types and indeed, they segregate well on the PCoA plot. Also, distinct separations of larvae and adults (both fecal and gut samples) were observed. This graphical output allows the researcher to see that combined, the adult microbiomes are different from the larval microbiomes and both are different from the soil matrix in which they are house and in the case of the larvae, feed. The difference between the soil samples and the others also points to the importance of inoculation by the rest of the environment and food sources in the life of the green June beetle.

Figure 10. Weighted UniFrac PCoA plot graphically describing the microbiome differences between the sampling sources. Soil (green squares), adult gut (red triangles), adult feces (blue circles), larvae (orange triangles).



4 Conclusions

There was some expectation that the higher concentrations of RDX and phenanthrene (i.e., 25 ppm) would elicit negative effects on development or survival of larvae which would be actively consuming the contaminated soils. However, there was no evidence of detrimental effects on the GJB larvae or their development into adults when compared to controls. Likewise, little to no perturbation of the larval or adult microbiome was observed. There are numerous studies that show toxicity of explosives but RDX is not always considered highly carcinogenic or toxic to mammals, arthropods, or bacteria (Abadin et al. 2012; Drzyzga et al. 1995). Interestingly, despite phenanthrene being considered highly toxic and being one of the EPAs 16 priority pollutants (Kibria 2019; Nota et al. 2009; Suszek-Lopatka et al. 2016), there was no apparent effect of this compound on GJBs in our study. The green June beetle (and its close relatives) may be a useful model for future lab studies as it is cosmopolitan throughout most of the U.S., is not an invasive species that requires permitting, is docile and easy to handle, breeds prolifically, appears to have a relatively stable microbiome, and is very hardy.

References

Abadin H, Smith C, Ingerman L, Llados FT, Barber LE, Plewak D, Diamond GL (2012) Toxicological profile for RDX. U.S. Department of Health and Human Services,

Caporaso JG et al. (2010) QIIME allows analysis of high-throughput community sequencing data Nat Methods 7 doi:10.1038/nmeth.f.303

Caporaso JG et al. (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms ISME J 6:1621-1624

Caporaso JG et al. (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample PNAS 108:4516-4522

Crocker FH, Indest KJ, Fredrickson HL (2006) Biodegradation of the cyclic nitramine explosives RDX, HMX, and CL-20 Appl Microbiol Biotechnol 73:274-290

Crocker FH, Thompson KT, Szecsody JE, Fredrickson HL (2005) Biotic and abiotic degradation of CL-20 and RDX in soils Journal of Environmental Quality 34:2208-2216

Drzyzga O, Gorontzy T, Schmidt A, Blotevogel KH (1995) Toxicity of explosives and related compounds to the luminescent bacterium *Vibrio fischeri* NRRL-B-11177 Arch Environ Contam toxicol 28:229-235

Howe WL, Campbell WV (1953) A simple method for rearing green June beetle larvae. Agriculture Research Administration, Bureau of Entomology and Plant Quarantine

Jayamani I, Manzella M, Cupples A (2013) RDX degradation potential in soils previously unexposed to RDX and the identification of RDX-degrading species in one agricultural soil using stable isotope probing Water 224 doi:10.1007/s11270-013-1745-4

Johnson D (2009) Green June beetles:The wine makers of the insect world.

Jung CM, Crocker FH, Eberly JO, Indest KJ (2011) Horizontal gene transfer (HGT) as a mechanism of dissemination of RDX-degrading activity among Actinomycete bacteria J Appl Microbiol 110:1449-1459

Just S, Mondot S, Ecker J, al e (2018) The gut microbiota drives the impact of bile acids and fat source in diet on mouse metabolism Microbiome 6 doi:https://doi.org/10.1186/s40168-018-0510-8

Kibria G (2019) Oil spill and oil pollution- a threat to wildlife, fisheries, seafood security and ecosystems. https://www.researchgate.net/publication/333091943_Oil_Spill_and_Oil_Pollution A_Threat_to_Wildlife_Fisheries_Seafood_Security_and_EcosystemS.

Lu Y-F, Lu M (2015) Remediation of PAH-contaminated soil by the combination of tall fescue, arbuscular mycorrhizal fungus and epigeic earthworms Journal of Hazardous Materials 285:535-541 doi:http://dx.doi.org/10.1016/j.jhazmat.2014.07.021

Lundin JI, Riffell JA, Wasser SK (2015) Polycyclic aromatic hydrocarbons in caribou, moose, and wolf scat samples from three areas of the Alberta oil sands Environmental Pollution 206:527-534 doi:http://dx.doi.org/10.1016/j.envpol.2015.07.035

Nota B, Bosse M, Ylstra B, van Straalen NM, Roelofs D (2009) Transcriptomics reveals extensive inducible biotransformation in the soil-dwelling invertebrate *Folsomia candida* exposed to phenanthrene BMC Genomics 10:1-13

Olsena I, Yamazakib K (2019) Can oral bacteria affect the microbiome of the gut? J Oral Micrbiol 2019:1586422

Shukla SP, Sanders JG, Byrne MJ, Pierce NE (2016) Gut microbiota of dung beetles correspond to dietary specializations of adults and larvae Mol Ecol:1-14

Sun K, Liu J, Gao Y, Sheng Y, Kang F, Waigi MG (2015) Inoculating plants with the endophytic bacterium Pseudomonas sp. Ph6-gfp to reduce phenanthrene contamination Environmental Science and Pollution Research 22:19529-19537 doi:10.1007/s11356-015-5128-9

Suszek-Lopatka B, Maliszewska-Kordybach B, LKlimkowicz-Pawlas A, Smreczak B (2016) Influence of temperature on phenanthrene toxicity towards nitrifyingbacteria in three soils with different properties Environ Pollut 216:911-918

USEPA USEpa (2003) June bugs invade links; Army wins battle, loses war, retreats, finally wins with new tactics vol EPA/903/F-03/005. Philadelphia, PA

USEPA USEPA (2019) Phenanthrene Fact Sheet.

Vishniac HS, Johnson DT (1990) Development of a yeast flora in the adult green June beetle (*Cotinis nitida,* Scarabaeidae) Mycologia 82:471-479

Yuan M (2010) The preparation and analysis of polycyclic aromatic hydrocarbons in meat by GC/MS vol http://www.perkinelmer.com/PDFs/downloads/APP_PAHinMeatbyGCMS.pdf. Shelton, CT

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
November 2020	Final	

4. TITLE AND SUBTITLE

Response of the Green June Beetle and its Gut Microbiome to RDX and Phenanthrene

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611102AB2

6. AUTHOR(S)

Carina M. Jung, Matthew Carr, Eric Fleischman, and Chandler J. Roesch

5d. PROJECT NUMBER
18-040

5e. TASK NUMBER
A1170

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

U.S. Army Engineer Research and Development Center
Environmental Laboratory
3909 Halls Ferry Road
Vicksburg, MS 39180

ThermoFisher Scientific
Eugene, OR 97402

8. PERFORMING ORGANIZATION REPORT NUMBER

ERDC/EL MP-20-10

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

US Army Corps of Engineers
Environmental Quality Program
Washington, DC 20314

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

Originally published in the International Journal of Environmental Science and Technology on 10 October 2020.
https://doi.org/10.1007/s13762-020-02960-1
ERDC – EQI - 6.1 Military Direct Program

14. ABSTRACT

Green June beetles are a cosmopolitan pest in the United States. Adults are voracious consumers of tree and vine fruit, while their larvae can damage and inadvertently consume root systems, particularly those of grasses, as they move through the soil and forage for detritus. Larvae in-gest and process large volumes of soil while in the process of feeding. Due to their intimate contact with the soil it was hypothesized that soil contaminants that are known animal toxins would perturb the larval and affect their overall health and survival. Studies of this kind are important contributions to the development of new model organisms and our understanding of interactions between the environment, contaminants, gut microbiome, and animal development, health, and survival. It is important to continue to develop relevant model organisms for monitoring toxicity as regulations for working with vertebrates becomes more prohibitive. In this study green June beetle larvae were exposed to RDX and phenanthrene throughout their entire soil-bound development, starting within the first few days of hatching through to their emergence as adults. The overall findings included that even at high concentrations, RDX and phenanthrene (25 ppm) exerted no significant effect on body weight or survival. Also, there was little apparent effect of RDX and phenanthrene on the bacterial microbiome, and no statistical association with measurable health effects. Nevertheless, the green June beetle is an interesting model for soil toxicity experiments in the future as is it easy to collect, house, and handle.

15. SUBJECT TERMS

Beetle, RDX, Phenanthrene, Microbiome

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (include area code)
Unclassified	Unclassified	Unclassified	SAR	24	